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THE CHICAGO MEDICAL SCHOOL
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EXPERIMENTAL LATHYRISM*

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Human lathyrism has been known since the time of Hippocrates, who tells of patients having weakness of the legs and pain in the knees as the result of living on diets consisting largely of legumes or peas. The early history of lathyrism has been comprehensively reviewed by Schuchardt[1]. Stockman[2] has perhaps given the best description of its symptomatology.

The symptoms consist of a weakness of the legs and loins; the muscles are tremulous when weight is put on them, there is a dragging of the legs, increased reflexes and more or less inability to walk. In milder cases the patient can get about with or without the aid of a stick. More severe cases are unable to walk or stand, or can only do so with the aid of one or two long sticks. In extreme cases the patient is forced to progress on hands and feet in a sitting posture. The symptoms seem to be referable to central nervous system damage.

The term *lathyrism* came into use during the latter part of the eighteenth century when it became rocognized that this condition could be attributed specifically to the ingestion of legumes belonging to the genus *Lathyrus*.. Human lathyrism has been especially common in Indian and Algeria, its incidence in some districts of India having reached 6-7% of the entire population[2]. Small local outbreaks have also occurred throughout northern Africa, southern Europe, and Asia. The species of *Lathyrus* responsible for most outbreaks of lathyrism are *L. sativus* (chickling vetch), *L. cicera* (flat-podded vetch), and *L. clymenum* (Spanish vetchling).

Unfortunately, the term *lathyrism* is also used to refer to apparently unrelated conditions brought about by the consumption of other species of *Lathyrus*. The species of *Lathyrus* which cause human lathyrism are non-toxic to rats even when fed as the sole food for prolonged periods[3,4]. The condition in the rat which is commonly referred to as "lathyrism" bears little or no resemblance to human lathyrism. It appears to involve specifically the connective tissues. Because this syndrome is produced in the rat by the feeding of *Lathyrus odoratus* (sweet pea) seeds, the term odoratism has been proposed[5] and has received some support in the literature[6,7].

ODORATISM

Odoratism was first produced in the rat about 25 years ago by Geiger, Steenbock, and Parsons[8]. They fed diets containing the ground seeds of the flowering sweet pea *Lathyrus odoratus*, a plant which is commonly grown in this country for ornamental purposes. These diets

* This paper is intended to be a survey of the subject rather than a detailed review of the literature.

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produced marked skeletal changes, consisting of thickening and deformation of the long bones, spinal curvatures in the thoracic region, sternal curvatures, misshapen ribs, and enlargement of the costochondral junctions. The rate of growth was depressed. Hernias occurred in 25% of the young rats receiving a 50% level of sweet peas. In a few animals the hind legs became completely paralyzed. The authors called the condition "lathyrism."

Three other species of *Lathyrus* are known to give rise to a syndrome in the rat which is identical to that produced by *Lathyrus odoratus*. These are *L. tingitanus*[9], *L. pusillus* (the Singletary pea)[10], and *L. hirsutus* (the Caley pea)[9]. The use of the latter two species as winter crops in the Gulf states of this country has led to some cases of poisoning in livestock[9,11]. Certain other species of *Lathyrus*, such as *L. sylvestris* (the flat pea), while highly toxic to rats[9,12], give rise to a syndrome which is quite different from odoratism[9,13].

The toxic factor. The isolation of a crystalline substance capable of producing the symptoms of odoratism was first reported by Dupuy and Lee[14], who isolated the toxin from Singletary peas. Shortly thereafter, the isolation of active crystals from sweet peas was announced from two different laboratories[15,16]. Schilling and Strong determined the chemical structure of the toxic compound and showed it to be beta-(N-gamma-L-glutamyl)-aminopropionitrile[17,18]. (Fig. 1). It was soon recognized that the toxic properties resided in the beta-aminopropionitrile portion of the molecule and that the gamma-glutamyl residue did not contribute to its activity[16,19,20,21,22]. The toxic substance present in the Singletary pea has recently also been shown to be glutamyl aminopropionitrile[23].

A number of compounds which are chemically related to beta-aminopropionitrile (BAPN***) have been tested to determine their ability to produce similar skeletal changes[21,22,23]. Of these,

SUBSTANCES CAUSING SKELETAL CHANGES TYPICAL OF ODORATISM

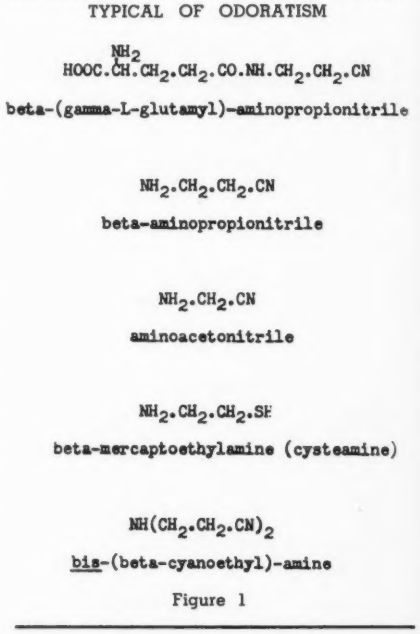

Figure 1

only a few were found to be active. Aminoacetonitrile was the most active, giving rise to extremely severe skeletal lesions in 2 weeks when fed to rats at a level of 0.2% of the diet[21,23]. Bis-(beta-cyanoethyl)-amine was much less effective than BAPN in bringing about skeletal changes[21,22,23], but produced, in addition, symptoms of central nervous system disturbance[22]. In our own laboratories it was found that beta-mercaptoethylamine, when fed at certain levels, was also capable of producing skeletal lesions which are grossly very similar to those found in odoratism[19]. This is an extremely interesting finding for two reasons: first, mercaptoethylamine occurs naturally in the body as the terminal group of Coenzyme A; second, it is the only toxic substance in this group which is not a nitrile.

Formulae for the substances which give rise to skeletal lesions resembling those found in odoratism are shown in Fig. 1.

Substitution of a hydroxy for the amino

*** "BAPN" will be used as an abbreviation for B-aminopropionitrile throughout this paper.

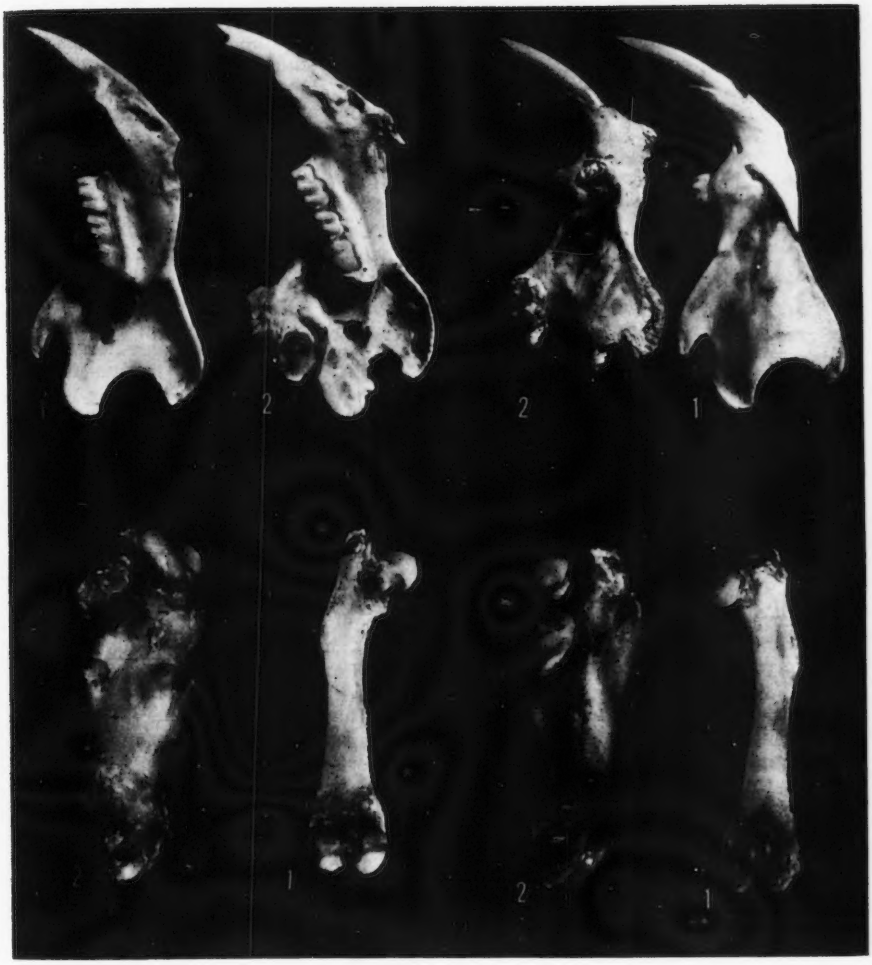

FIGURE 2

(From Dasler, W.: Proc. Soc. Exp. Biol. Med., *85*:485, 1954.) TOP ROW: Medial and later aspects of mandibles from rats receiving 50% sweet peas for 21 days (2) from their pair-fed controls (1). BOTTOM ROW: Opposite aspects of femur from rat receiving 50% sweet peas for 21 days (2) compared to femur of its pair-fed control (1).

group in BAPN or substitution of a methyl or acetyl into the amino group resulted in the loss of toxicity[22]. Methyleneaminoacetonitrile, however, was at least as toxic as aminoacetonitrile[24].

Garbutt and Strong have developed an analytical method for the determination of BAPN in biologic materials[25]. They found BAPN to be present in the seeds of those species of Lathyrus that give rise to odoratism. It was absent from a variety of other legume seeds, including edible garden peas, soybeans, alfalfa, and those species of *Lathyrus* that are responsible for human lathyrism.

Skeletal lesions. Fig. 2 illustrates the spectacular skeletal changes that take place in weanling rats fed a diet con-

taining 50% sweet peas for 21 days. The exostoses on the mandibles can be palpated within one week in living rats receiving 30-50% sweet peas in their diets. Even more drastic malformations can be produced by feeding higher levels of BAPN, either free[21] or in the form of one of its salts (Fig. 3). Scolioses. kyphoses, eternal curvatures, and thoracic cage deformities which may assume fantastic proportions occur in 2 to 3 weeks in rats fed a diet containing 50% sweet peas.

Microscopic and roentgenographic studies of the bones of rats suffering from odoratism have shown that an osteoporosis occurs in these animals[5,9,26]. Dasler, however, has shown that, while a decreased mineralization of the skeleton does occur in weanling rats fed 50% sweet peas for 3 weeks, the decrease in mineralization is not drastic and does not begin to approach the severity of that found in experimental rickets[27]. The mineralization of the whole incisor, in fact, shows a slight but statistically significant increase. Serum calcium and phosphorus levels are also within normal limits[8]. These results, together with the fact that serum alkaline phosphatase levels are apparently unaffected both in adult[28] and in growing rats[6], support the view that odoratism does not involve a primary disturbance of calcium and phosphorus metabolism. The decreased mineralization *per se* must be entirely unrelated to the bone deformities since no such deformities are seen in rickets.

It was also found that both the proximal and the distal ends of the femurs pull away easily from the shaft at the epiphyseal lines[27]. This occurs much more easily than in the case of the rachitic femurs having a much lower ash content. There seems, therefore, to be a deficiency in some kind of cementing substance in the bone which can vary independently of the dregree of mineralization. It was also noted that the incisors of rats in which odoratism had been produced were much more easily extracted than those of control rats, indicating a change in the peridontal membrane.

Ponseti and Shepard have made intensive studies of the skeletal lesions[20]. Among the changes which they observed were loosening and detachments of the tendinous and ligamentous insertions. A widening and extensive disruption of the epiphyseal plates occurred, the cartilage matrix appeared to lose cohesion and the cartilage cells were grouped in clusters. The epiphyseal lesions gave rise to epiphyseal slippings. Other findings were: metaphyseal fibrous defects, detachments of the tibial tuberosity, subluxations and dislocations of the shoulder diastasis of the sacro-iliac joints, degeneration of the intervertebral discs and disc herniations. In some rats, changes in the femoral head resembled those of Legg-Perthes disease. In a few older rats, lesions resembling those seen in Paget's disease were observed. In practically all rats there was a detachment of the periosteum followed by new-bone formation.

While there is in the growing rat a general thickening of the long bones, exotoses are prominent, especially at points of muscle attachment. Hamre and Yaeger have reported changes in the femurs of *adult* rats fed diets containing 50% sweet peas[29,30,31]. Twenty-four hours after the first feeding, increased mitosis of the fibroblasts of the inner layer of the periosteum was present, but at the point of muscle attachment only. Mitoses increased in number until the sixth or seventh day producing a cellular mass large enough to be identified grossly. Exostoses were produced only if the

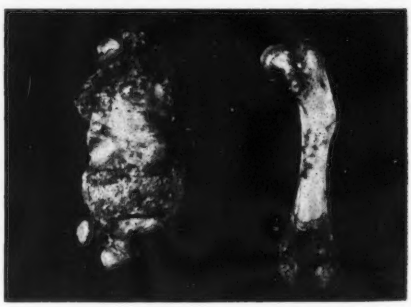

FIGURE 3

Flexor aspect of right femur from weanling rat receiving a complete stock diet containing 0.8% BAPN acid fumarate (equivalent to 0.30% free BAPN) for 14 days, compared to a normal femur.

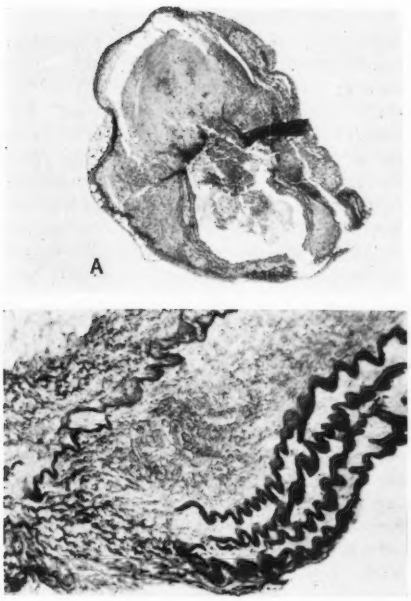

FIGURE 4

Aortic lesions in mice fed sweet peas or BAPN. *A* (X30) Transverse section of thoracic aorta showing separation of elastic fibers, medial dissection, and aneurysm formation with thrombosis. *B* (X400) Area of medial dissection showing fragmentation and lysis of elastic fibers.

bone at the point of exotosis formation was also subjected to muscle tension. Exotoses failed to appear at the insertions of muscles which had been sectioned surgically.

An increased uptake of radioactive sulfate and phosphate has been reported to take place in the femur shafts of rats poisoned with BAPN[32]. The authors explained this on the basis of an enhanced accretion of cartilage and bone, probably secondary to the slipping of muscular insertions.

Vascular changes. Ponseti and Baird were the first to report the occurrence of dissecting aneurysms of the aorta in rats fed sweet peas[26]. Aortic aneurysms have since been repeatedly observed, both in rats fed sweet pea diets[13,20,33,34,35] and in rats receiving BAPN[13,21,22,36]. They occur most frequently in the thoracic aorta, especially in the arch, and more rarely in the abdominal aorta. Death in these animals is commonly due to rupture of the aneurysm.

Microscopic studies of the aorta[33,34,35] have demonstrated a medial edema followed by fragmentation of the elastic fibers. The elastic fibers show a generalized disintegration with interruption of continuity. Dilatation or dissecting medial hemorrhage may follow. Reparative fibroblastic proliferation occurs in the damaged area.

A marked increase both in the metachromatic staining and in the Schiff-positive mucopolysaccharides has been reported[34]. Whether this increase in mucopolysaccharides precedes or is coincident with the disruption of the elastic fibers is not clear. The first change in the media must certainly be a chemical change however, causing altered osmotic relationships and thus giving rise to the initial "edema" or spreading apart of the elastic fibers.

Although gross aneurysms have been observed only in the aorta, Walker and Wirtschafter have found that lysis of elastic fibers and hyperplasia of fibroblasts occur also throughout the pulmonary arteries and even in the coronary arteries[35].

We have recently found that similar changes occur also in the aortas of mice[37] (Fig. 4).

Neurological effects. A phenomenon frequently seen in rats suffering from moderate to advanced odoratism is paralysis of the rear legs. This has been ascribed by some to a specific neurologic effect of the toxin[5,38]. Others haxe felt that the paralysis could be explained by mechanical injury to the spinal cord resulting from the severe kyphosis of the thoracic spine[6,7,9]. The present author has recently arrived at the conclusion that neither of these hypotheses adequately explains the various neurologic manifestations of odoratism.****

The paralysis occurring in odoratism

****The author is grateful to Dr. R. V. Milliser of the Department of Pathology of The Chicago Medical School for very helpful discussions of the pathology of odoratism.

has invariably been described as a spastic paralysis. In our own observations on literally hundreds of rats suffering from odoratism, we have seen only a completely *flaccid* paralysis of the rear legs. The musclar "spasticity" described in the literature is also probably not a true spasticity. The jerky movements and poorly coordinated gait probably result from the awkward posture and limited range of movement occasioned by the tremendous overgrowth of bone about the joints, especially the hip joint. Contributing factors may also be the pain following the periosteal detachments and the slipping epiphyses, as suggested by Ponseti and Shepard who also question the presence of spasticity[20].

While it is true that mechanical injury to the spine does occur in the thoracic region (in an occasional rat the spinal cord is found to be completely severed at the point of angulation of the spine [6,20]), injury to the spine in this area should result in a spastic paralysis. Furthermore, Vivanco and Diaz[5] performed decompression laminectomies in rats before the appearance of kyphosis without any effect on the development of lameness and paralysis.

In our laboratory we have observed the following additional symptoms of odoratism which appear to be neurologic in nature: a high incidence of paraphymosis, especially in the chronic or semichronic conditions; urinary retention, which may be so extreme in some groups of rats that death not infrequently results from a ruptured urinary bladder; atony of the colon, which may result in severe fecal impaction.

All these symptoms — paralysis of the rear legs, paraphymosis, urinary retention, and atony of the colon — may occur independently of each other or in any combination of two or more. They therefore probably do not arise from a single neurologic lesion. The most logical explanation for these facts seems to be a mechanical compression of one or several of the lower spinal nerves resulting from stricture of spinal foramina. This is not unlikely in view of the severe overgrowth of bone which is found throughout the whole skeleton.

Prenatal effects. It has been established that the severity of the symptoms of odoratism, after a given period of feeding, varies with the age of the rat at the time the feeding is started. The younger animal when placed on a sweet pea- or BAPN-containing diet, the more drastic are the skeletal and the vascular changes. Recent reports regarding the effects of the toxin on the features of pregnant rats fed sweet peas or BAPN are therefore of interest.

Marked kyphosis of the thoracic spine and changes in the aorta similar to those seen in weanling rats, *i.e.*, edema of the media, fragmentation of elastic fibers, dissecting aneurysms, and ruptured aortas were found in fetuses of mothers receiving a 50% sweet pea diet throughout the gestation period[39,40]. No viable young were born. Similar effects were seen when the sweet pea diet was only fed toward the end of gestation. When sweet pea or BAPN-containing diets were fed to pregnant rats for the first 16 days of pregnancy and then replaced by normal diets, no harm to the fetuses resulted; normal young were born[39,41]. If the feedings of these materials were continued even for one more day, death of almost all fetuses occurred. As Stamler has pointed out, it is probably significant that the fetus is susceptible to the toxic action of BAPN only during the period of rapid mesodermal growth and differentiation of late intrauterine life, although differences in placental permeability toward the toxin might also account for the difference in susceptibility shown during early and late fetal life[39].

Marked protection against fetal death and resorbtion has been afforded by the daily administration of estrone and progesterone to pregnant rats[42]. In this connection, it is noteworthy that pregnant females fed 50% sweet pea diets have been reported to show no osseous changes throughout the normal gestation period (22 days), whereas roentgenograms of adult non-pregnant females revealed osseous changes on the 14th day after the feedings were begun[41].

Effect in animals other than the rat. It has been reported that white mice

are completely resistant to the toxic action of diets containing 50% sweet peas[43]. Recent studies in our laboratory, however, reveal that while mice are indeed more resistant than are rats, similar skeletal and aortic changes are produced by diets containing sweet peas or BAPN[37]. Thus, we have observed spinal curvatures, exostosis formation, aortic aneurysms, fragmentation and lysis of the elastic fibers of the aortic media with concomitant fibroblastic proliferation, and paraphymosis in mice receiving such diets. Paralysis and urinary retention have not been observed.

Studies of the effect of BAPN upon the development of the larvae of the South African toad have been reported[44]. BAPN or powdered sweet pea seeds were added to the aquarium water in low concentrations. Severe limb deformities, subcutaneous hemorrhages, and death occurred. A characteristic deformity was the dislocation of the knee and ankle joints. Microscopic examination showed a loosening of leg muscles, tendons, and ligaments. Dislocations, atrophy of the leg muscles, and loosening of tendinous insertions also occurred in the regenerating hind legs of newts[44]. The same report also described the effects of BAPN on the developing chick embryo. Sublethal levels affected particularly the development of the legs, causing a bending of the femurs.

Glutamyl-BAPN (the natural form of the sweet pea toxin) has been shown to affect also the development of the embryonic salamander[45]. Embryos treated with this substance had distorted forelimbs, shortened mandibles and tumors of the notochord. The suggestion was made that the notochordal tumors arose "as a consequence either of a focal degeneration of the notochord sheath, leading to secondary proliferative ecchordoses or prolapses, or of a direct stimulation to proliferation of the notochordal cells."

In addition to these published reports, studies are underway in several laboratories on the effects of BAPN on a number of avian and mammalian species[46].

Protective substances. Many screening tests have been carried out in our laboratory in an attempt to find substances having a protective action against the sweet pea toxin[47,48]. It was anticipated that the finding of such substances might offer a clue to the mode of action of the toxin.

Certain proteins were found to be partially protective when fed at high levels[47,48]. These are, in order of decreasing effectiveness: casein, lactalbumin, caesin hydrolysate (enzymatic), and gelatin. Although the protective actions of casein and of gelatin have been questioned[24], these findings have been confirmed not only by repeated observations in our own laboratory but also by observations in other laboratories[36,38,49]. Zein, a corn protein, has no protective activity[38,48].

Of a wide variety of pure compounds, including amino acids, carbohydrate components of mucopolysaccharides, vitamins, substances chemically related to BAPN, and other miscellaneous chemicals, only L-cysteine (but not L-cystine) and L-glutamine (but not L-glutamic acid or glutamate) proved to be partially protective[48]. Cysteine and glutamine, when fed together, were more effective than was either alone. *Complete* protection by dietary supplements has never been observed. It may be, of course, that the level of the toxin in the test diets has simply been too high for *any* supplement to give complete protection.

Injections of cortisone acetate (0.5 mg./day) and of thyroxine had no protective effect[13,48]. Injections of growth hormone increased the rate of development and the severity of the skeltal lesions[13]. In this connection, attention should again be called to the apparent protective effect of the placenta against the development of osseous lesions in the pregnant female[41] and to the protection afforded the fetus by estrone and progesterone when injected into the mother[42].

It has been our experience that variations in the diet with respect to levels of BAPN or of sweet peas, or with respect to supplementation with protective agents, cause variations in the incidence of certain of the manifestations of odo-

ratism. Thus, paraphymosis is more common in the more slowly developing condition, whether the decreased rate of progress of the disease is caused by decreased levels of the toxin or by the presence of protective agents[13], *i.e.*, paraphymosis is more common in the semichronic than in the severely acute conditions. Others have noted an increase in the incidence of paralysis when the rate of progress of odoratism was slowed by high levels of casein[38]. Methionine, which exerts no protective action, has been reported to increase the incidence of aortic rupture[36].

Other considerations. Dasler has reported that the skin of rats with advanced odoratism was extremely fragile and tore easily when a hypodermic needle was inserted subcutaneously[6]. Total skin weights were reduced by 30% when compared on the basis of surface area to those of pair-fed control rats. No microscopic studies of skin have as yet been reported.

Toxic effects are thus known to occur in the skin, the skeleton, the vascular system, and the peridontal membrane. Histologic sections of other abdominal and thoracic organs have shown no abnormalities[26]. It appears, then, that odoratism affects primarily connective tissues and probably involves some aspect of connective tissue metabolism.

Most studies seem to indicate that a deficiency in collagen formation does not exist. The average collagen content of the femurs from rats with advanced doroatism was found to be the same as that of femurs from pair-fed control rats[50], while the collagen content of skin was significantly increased over control levels[13]. Electro-microscopic studies of the fibers of the tail tendon have been reported to show no structural abnormalities in the collagen fibers[20]. Furthermore, damaged areas in the aortic media seem to show increased collagen formation in an attempt at repair. However, a decrease in the hydroxyproline content of developing connective tissue from croton oil pouches has been reported in rats fed BAPN[51]. This was correlated with a delayed collagen synthesis which was observed microscopically in such pouches.

The uptake of radioactive sulfur by the thoracic aorta, and presumably by chondroitin sulfate, has been reported to be the same in experimental as in control rats[24]. The authors conclude from this, that the sulfur content of the chondroitin sulfate is probably not greatly altered in odoratism. As mentioned above, there is an increase in the metachromatic-staining and in the Schiff-positive mucopolysaccharides of the media of the aorta[34]. Preliminary hexosamine determinations on extracts of skins and femurs also do not indicate a deficiency in mucopolysaccharide formation[13]; in fact, increased mucopolysaccharide formation in bone may be indicated by the finding of increased moisture and hexosamine levels in the femurs[13].

Isotopically labeled BAPN administered to rats has also not revealed the site at which the toxin acts[52].

The specific metabolic defect in odoratism is as yet undetermined. It apparently involves some aspect of connective tissue metabolism with the result that the binding power of the ground substance may be decreased.

Possible relation to human disorders of unknown etiology. The dissecting aneurysms of the aorta which occur in rats suffering from odoratism are grossly and microscopically similar to cases of medial degeneration of the aorta in humans[20]. Several authors have pointed to a possible relation between these experimentally produced aneurysms and the cardiovascular lesions commonly seen in *arachnodactyly* (Marfan's syndrome) [53,54,55,56]. Sloper and Storey first suggested that the diverse lesions seen in Marfan's syndrome result from a basic disturbance in the ground substance[57]. Other authors have given credence to this hypothesis[53,54].

Ponseti and McClintock have related the lesions found in biopsy specimens from patients with *early slipping of the upper femoral epiphysis* to similar lesions occurring in rats fed BAPN[58]. The "primary lesion in such cases is loss of co-

hesion of the cartilage matrix, presumably due to an alteration in the chemical composition of the ground substances." The authors suggest that the underlying cause of epiphysiolysis in humans is a metabolic disturbance.

Ponseti has also demonstrated a similar disruption of the epiphyseal plate in *Legg-Perthes disease*[59]. He emphasized the fact that in this condition similar lesions occur in epiphyses other than those of the femoral head. Ponseti and Shepard have discussed the possible relation of *idiopathic scoliosis* in humans to sweet pea toxicity in rats[20]. The distribution of various nitrogen- and sulfur-containing constituents in the urine of children with idiopathic scoliosis has been reported to be altered from the normal[60], but is similar to that found in patients with early slipping of the upper femoral epiphysis[58], a finding which seems to relate these two diseases. Adult rats fed sweet pea diets for several months developed joint lesions similar to those of *osteo-arthritis* in human patients[20]. Ponseti and Shepard discuss the similarities of a number of *other human skeletal pathologies* of unknown etiology to the skeletal abnormalities seen in rats fed sweet pea diets[20].

SUMMARY

When the seeds of *Lathyrus odoratus* (the sweet pea) or of several other species of *Lathyrus* are fed to rats, they give rise to a form of experimental lathyrism which has been termed odoratism. Other species of *Lathyrus* produce symptoms of toxicity in humans or in rats which are quite dissimilar to those of odoratism.

The substance responsible for odoratism is beta-aminopropionitrile (BAPN) which occurs in certain Lathyrus seeds as the glutamyl derivative. Aminoacetonitrile and mercaptoethylamine produce skeletal changes similar to those produced by BAPN.

It appears likely that BAPN interferes with some phase of connective tissue meabolism as a result of which the binding power of the ground substance is decreased. This may be the basis for its varied effects in the experimental animal: fragility of the skin, medial degeneration in the aorta, epiphyseal slipping, and periosteal detachments and loosening of tendinous insertions resulting in exostoses and periosteal new-bone formation. A number of human diseases of unknown etiology seem to involve a similar disturbance of connective tissue metabolism.

Partial protection against sweet pea toxicity can be obtained by the feeding of high levels of certain proteins: casein, lactalbumin, casein hydrolysate, and gelatin. Zein gives no protection. Cysteine (but not cystine) and glutamine (but not glutamate) are also partially protective. When cystine and glutamine are fed together, somewhat better protection is obtained than when either is fed alone.

That hormonal factors may also be involved in odoratism, is indicated by the findings that pregnancy protects adult rats against the development of osseous lesions and that the administration of estrone and progesterone to pregnant females has a protective action against fetal death and resorption.

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TRANSCELLULAR TRANSPORT MECHANISMS

HANS ELIAS, Ph.D.[1] and SHELLY BORTNER, A.B.[2]

The passage of substances through cells against osmotic pressure is among the physiological phenomena that appear to us most mysterious at this time. As with many other problems, we hope that this phenomenon will become explicable in the future. The electron microscope has brought us a step closer to its solution.

In many cells, particularly in those where active secretion or excretion is known to occur, double membranes, 100-400 Angstroms thick in total (fig. 1), have been described[1]. In certain cells which have a secretory function, e.g. exocrine pancreas,[2, 3, 4] as well as in chloroplasts,[5] these membranes are concentrically arranged around a nucleus and fill almost the entire cell[6, 7]. Sjostrand and Rhodin,[8] and Rhodin[9] have shown extensions of the outer cell membrane deep into the interior of the cells of the proximal convoluted tubules of the kidney (fig. 2). These double extensions of the outer cell membrane have the same electronmicroscopic structures as intracellular membranes known to exist in other organs. These projecting double membranes divide the basal part of these cells into compartments in a honeycomb-like pattern. In each of these compartments a long mitochondrion is located (described by light microscopists as the rods of the basal zone). These mitochondria are surrounded by a double membrane from which transversely directed double membranes project into the interior of the mitochondrion, dividing it into transverse compartments. Rhodin has also demonstrated many small single membranes in the cytoplasm of these cells. The extensions of the brush border which are also lined by the same cell membrane are so closely in contact with each other that they appear to be separated, in horizontal sections, by double membranes.

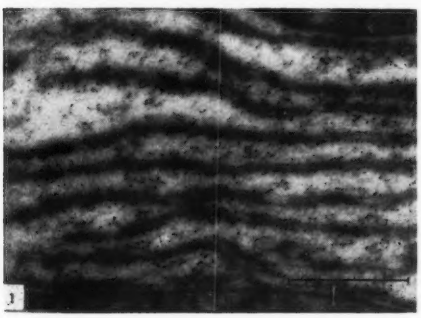

Figure 1. Double membranes, as seen in the liver cell. (Electronmicrogram by Dr. Don W. Fawcett.)

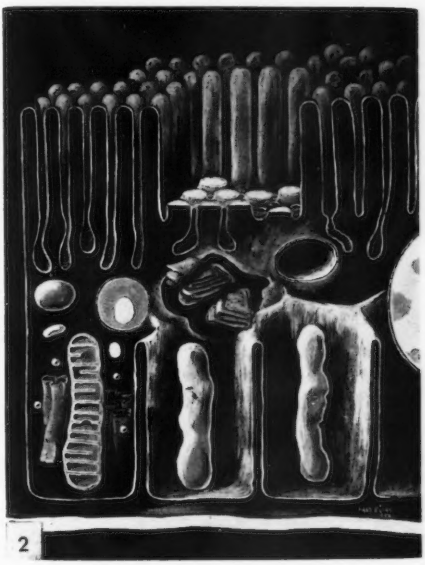

Figure 2. Stereogram of a cell from the proximal convoluted tubule, redrawn after Rhodin.

In liver cells, which have an excretory function and also perform osmotic work, these membranes are found in piles[10, 11, 12] (fig. 3), chiefly in regions around bile canaliculi (fig. 4). They do not fill the entire cell. It has been pointed out[10] that membranes which are placed at cer-

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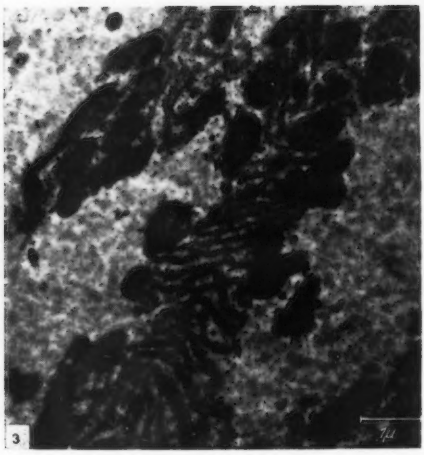

Figure 3. **Membranes in pile formation, as seen in liver cells (electronmicrogram by Dr. Don W. Fawcett).**

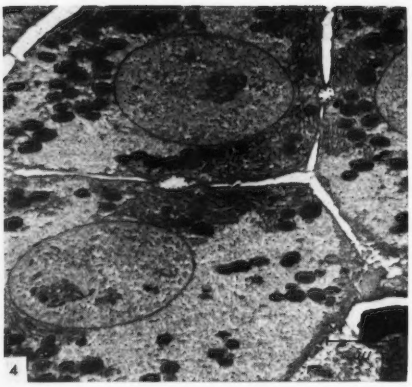

Figure 4. **Membranes observed in the regions around bile canaliculi. (Electronmicrogram by Dr. Don Fawcett.**

tain locations in the cytoplasm must have an influence upon the movement of substances within the cell since, because of their varied shape and arrangement, they must provide an obstacle to the simple diffusion of substances. On the other hand, they may actively transport substances against osmotic pressure.

There are cells in which such double membranes are entirely absent, and which are also known to permit the passage of substances through them. These are cells which do not perform osmotic work, e.g., endothelial cells. Best known among endothelial cells are the specialized cells of the renal glomerulus. The renal glomerulus has been shown to consist of a branched sheet, the lamina vascularis glomeruli (Elias[13]). This lamina probably consists of a syncytial mass of "endenchymal" cells, tunneled by blood channels and surrounded by the basement membrane, upon which the cells of the visceral epithelium of Bowman's capsule (termed "podocytes" by Hall and "epicytes" by Kuhlenkampff) are sitting. Figure 5 is a stereographic representation of a small portion of the lamina vascularis glomeruli. Three endenchymal nuclei (bottom of picture), are surrounded by a mass of cytoplasm. At left we see a blood channel within the endenchyma, the cytoplasm of which is attenuated to form a cribriform layer, called lamina fenestrata (Hall, 1953). Figure 6 shows the entire filtration membrane, emphasizing the structure of the lamina fenestrata. Oberling, Gauthier, Magenau and Bernard,[14] Hall,[15] and Peace and Baker[16] have demonstrated pores of 1000 Angstroms diameter which readily permit the passage of molecules of relatively large size. Betz[17] showed similar pores in the flattened cytoplasm of the endothelium in the zona fasciculata of the adrenal cortex, a location at which the passage of large-moleculed steroid hormones from the parenchymal cells into the blood is known to occur. The fact that such endothelial pores have not been described in other locations is to be ascribed to the circumstance that electron microscopy of microtome sections is a comparatively new method, and only a limited number of organs and tissues have as yet been submitted to this technique.

A third kind of cell through which passage of substances occurs, but not against osmotic pressure, is the above mentioned podocyte or epicyte found in the visceral epithelium of Bowman's capsule. Though Hall did not describe this circumstance, some of his electronmicrograms show very thin extensions of the cytoplasm of the podocytes. These membranes are only 400 Angstroms thick and

so delicate that one readily understands their apparent absence in many electronmicrograms. For even if such a membrane exists everywhere above the podocytic extensions, it must be expected that a formation of such extreme delicacy should disintegrate almost instantaneously after death and that even after fixation it would be destroyed during the embedding and cutting process. Figures 20 and 25 (the former by Hall, the latter by Reger) in a paper by one of us[18] show podocytic membranes, but not by far as well as in some of Hall's unpublished pictures.

One therefore is led to suspect that these cells cover the entire glomerulus, and that therefore the primary urine, after having been filtered through the glomerular basement membrane, still must pass podocytic cytoplasm. Hall believes that this passage occurs either through very large openings in the podocytes or through the spaces between podocytes before the urine enters into the space of the glomerular (Bowman's) capsule. It appears doubtful that such spaces between podocytes, as shown in electronmicrograms exist during life; on the other hand, the large holes which Hall demonstrated in the podocytes with the electron microscope are perhaps intra-podocytic vacuoles. And it may be suspected that the podocytes, in addition to their regulatory function, which they exert on the filtration rate by means of interdigitating processes or "pedicels" (Hall), also perform the function of pinocytosis, i.e. swallowing, mechanically, on their visceral side, the primary urine and expelling it on their external side into the glomerular capsule.

Physiologists have found that some hydrostatic or mechanical pressure (in addition to osmotic pressure) counteracts the glomerular blood pressure.

These forces are diagramatically illustrated in figure 7. Hall[15] claims that his findings on the glomerular wall account fully for the physiologically observed mechanical filtration process. Figure 8-B demonstrates Hall's concept of the filtration process. The primary urine passes through the large pores of the lamina fenestra (attenuated endenchyma) between the little feet (pedicels) of the podocytes. He also believes that these little feet by changing their shape, as indicated in figure 8-B can control the area available for filtration. When they contract (above) that area is relatively large. When they expand (below) they can block the filtration surface. Now, Hall believes that after the filtrate has passed between the pedicels, it has arrived in the space of the glomerular capsule.

If this were true, the filtration pressure (according to the physiologists) would be opposed by intracapsular hydrostatic pressure and by renal interstitial pressure (figure 7).

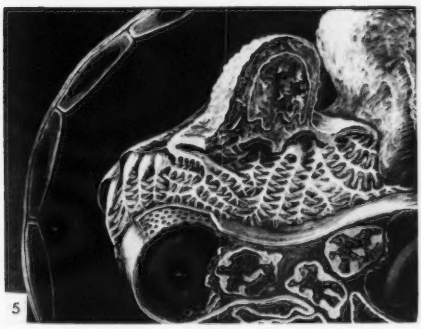

Figure 5.: Stereogram showing podocyte, *lamina vascularis glomeruli* and three endenchymal nuclei. (Courtesy G. D. Searle and Company.)

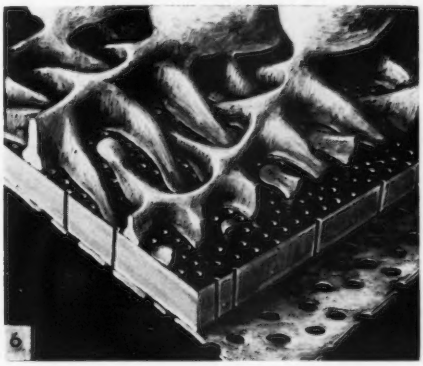

Figure 6. Stereogram showing at high magnification, pedicels of a podocyte and the entire filtration membrane, especially the *lamina fenestrata*. (Courtesy G. D. Searle and Company.)

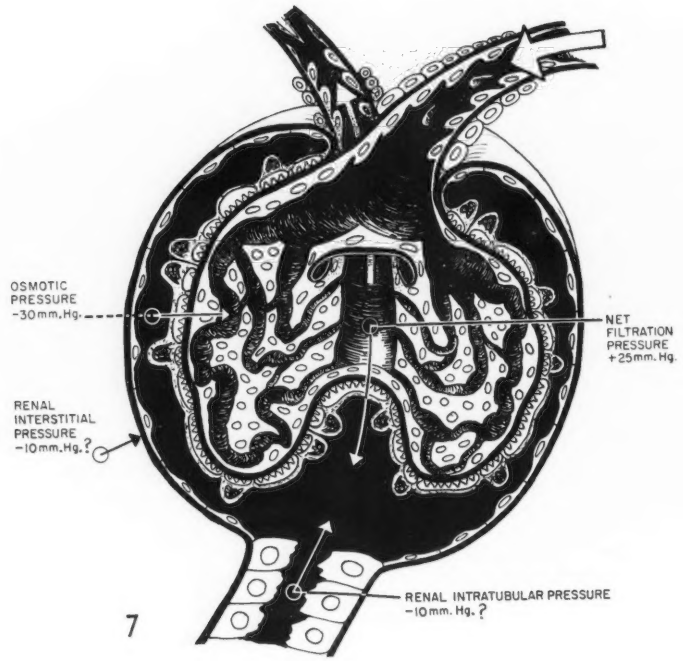

Figure 7. Diagram of a renal glomerulus illustrating the conventional views of forces at work in glomerular filtration.

But how does this pressure come about? Can it really exist? If interstitial pressure existed, this should fluctuate with the respiratory movements of the diaphragm. Measurements on this question are needed to demonstrate it. It is unlikely that intracapsular pressure exists. For not only does the ureter exert a pumping action, but the circular musculature of the minor calyces exerts a "milking" action. Further, reabsorbtion of water along the nephrons and the collecting tubules together with these muscular motions are likely to produce collectively a negative pressure in the glomerular capsule. Whatever positive hydrostatic pressure exists which may counteract the glomerular blood pressure, however slightly, is probably the pressure in the subpodocytic spaces, as schematically illustrated in figure 8-A.

The podocytes must therefore be equipped with a mechanism of liquid transport. This process might very well be pinocytosis.

Hence, the podocytes probably control glomerular filtration in two ways: first, by varying the width of their pedicels, and second, by temporarily retaining the filtrate in the subpodocytic space or by allowing its passage through their cell bodies.

Another area where transport of substances might occur by polarized pinocytosis and liquid expulsion is the trophoblast of *Ungulata* (hoofed animals). These animals have placentae of the epitheliochorial type where uterine "milk" is secreted into the uterine cavity by the uterine glands and by the uterine surface epithelium. The processes of the trophoblast cells which are in contact with the uterine wall and with the uterine lumen suggest that pinocytosis might occur in this tissue (figs. 9 and 10). Our

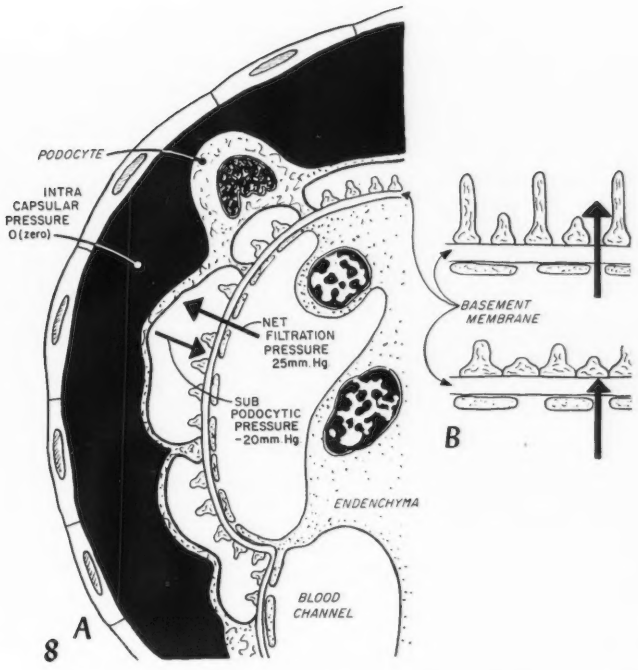

Figure 8. Diagrams of newer concepts of glomerular filtration

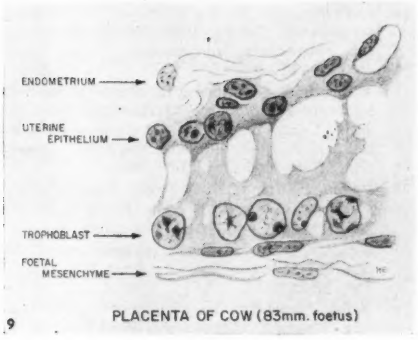

Figure 9. **Placenta of cow.**

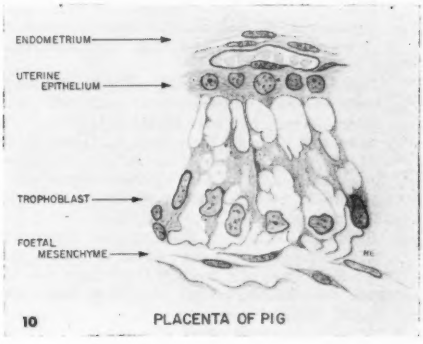

Figure 10. **Placenta of pig.**

studies of the placentae of goat, pig and cow lead us to the impression that uterine "milk" is transported across the trophoblast, within its intracellular vacuoles, from the distal surface of the trophoblast to its basal surface, until it is discharged by bursting of the vacuoles into the tissue spaces of the embryonic mesenchyme present in the chorionic villi. Preparations such as the ones shown in Figures 9 and 10 suggest the following process:

The uterine epithelium secretes a fluid which is pushed, in the form of large drops, into the superficial cytoplasm of

the trophoblast cells. Flat cytoplasmic extensions of the trophoblast cells grow around parts of these drops so that these become intracellular vacuoles. The vacuoles are transported by cytoplasmic movements toward the basal surface of the cell. Finally, they are separated from that surface only by a thin membrane of cytoplasm which ruptures and thus releases the fluid into the tissue spaces of the embryonic mesenchyme.

The human plasmoditrophoblast seems to engulf components of the maternal blood by phagocytosis and pinocytosis and to transport them into the embryonic mesenchyme.

In conclusion, it appears that three mechanisms may be at work when substances pass through the cells:

1) Simple diffusion through very large pores, such as in the endothelium of the renal glomerulus and the zona fasciculata of the adrenal cortex,
2) Pinocytosis, i.e., the active engulfing of liquid by cells and its expulsion on the opposite surface of the same cell, as in the trophoblast of certain mammals and perhaps in the podocytes of the renal glomerulus,
3) A not yet understandable process, most probably facilitated by intracellular double membranes.

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KIDNEY BIOPSY AS DIAGNOSTIC TOOL

AARON GROSSMAN, M.D.*

During the past decade many scientific disciplines have been developed and integrated which resulted in unveiling and unraveling the mystery of many disease processes. Not so, however, with the kidney. While inroads have been made in the physiology of this organ its many clinical disease processes and the pathological and morphological manifestations thereof, details of its anatomy remain unsolved, but not unchallenged. One of the many ways of learning more about the kidney is by biopsy. In 1951 Iversen and Brun[1] attempted this procedure with some success. Many of their failures were thought to be due to the technical difficulties arising when the biopsy was done in the upright position. Later, Kark and his colleagues[2] modified this technique and pointed out the added safety and ease if this procedure was done in the prone position. This procedure, in these investigators' hands, has proved a safe and poignant means of studying adult renal disease. Bjornaboe and his associates[3] described the glomerular changes found in adults with the nephrotic syndrome associated with amyloidosis or chronic glomerulonephritis and also in patients with so-called "pure" nephrosis. They also pointed out that the quantity of tissue generally obtained by kidney biopsy is sufficient to give a representative impression of the renal lesion when the renal lesions are diffuse. Muehrcke, a member of Dr. Kark's group, and Dr, Kark[4] have been particularly impressed with kidney biopsies as an aid in making an exact diagnosis in the nephrotic syndrome, in the so-called "Collagen Diseases,"[5] in Amyloidosis, Pyelonephritis and many other kidney lesions, where the clinical symptoms and ordinary laboratory tests are not too much help in making the diagnosis. Clinically, medical-renal disease in infants and children is also in a similar state of confusion. Even though people working with diseases of the kidney in this young age group do not quite agree that the concepts, pathogenesis, and etiology of medical-renal diseases in children is the same as it is in adults, it appeared very worthwhile to use this technique in children.

Serial Percutaneous Renal Biopsis offer a means of studying:

1. The morphologic changes during the acute phase of the illness
2. The influence of many therapeutic regimes—i.e., do steroids really influence morphologic changes in the nephrotic syndrome or is it more apparent than real?
3. The natural history of the disease, i.e., does one type of kidney lesion actually progress in time to a more serious one, or do the lesions as one sees them clinically appear morphologically as such without progression and then either heals, smolders, or succumbs.

An excellent intravenous pyelogram is essential to the successful aspiration of renal tissue. According to Caffey and Silverman[6] no preparation is necessary. The children are placed on an x-ray table in the usual way with compression and a minimum of 20 milliliters (for smaller children 2 to 5 years) and a maximum of 30 milliliters (for larger children 5 years and up) of contrast medium is injected slowly into the ante-cubital vein. We use Urikon,— and according to Caffey the trick is to give a lot of dye, and to this we can attest. Films are taken at regular intervals and each one is developed immediately.

Suffice it to say at this time that many laboratory tests are done to safeguard the patient. Severe azotemia, bleeding or coagulation defects and anemia are just a few of the conditions which are checked and which if abnormal, preclude the biopsy.

One half hour before the time of

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biopsy, the child is given .05 to 0.1 gm. sodium pentobarbital (nembutal). He is then placed in the prone position on a surgical cart with a small sandbag snugly under the abdomen, high enough so one can get one finger between the sandbag and the lowest rib. At the head of the cart, sits an assistant who plays and talks with the child. Blood pressure and pulse are taken every five to ten minutes. From the pyelogram the following measurements are made:

1. Middle of the spine to the lateral border of the kidney
2. Middle of the spine to a mark "X" which is the biopsy site, usually located at the lower pole of the kidney, because of its avascularity.
3. Superior iliac crest to the same biopsy site marked "X."

These measurements are then duplicated on the back of the patient and the same biopsy site is located. Sterile techniques are used; the patent draped and prepared. Incidentally, with thin children and even somewhat obese ones, in this position the kidney is easily palpable on deep inspiration. Over the biopsy site which is infiltrated with 1% Procaine, a thin exploring needle is carefully inserted little by little and only while the patient holds his breath in inspiration. When the needle is within the kidney, it will sway in a large arc with respirations. The depth is thus measured and marked and the needle withdrawn. The biopsy needle is then inserted in the same way, to the measured depth, and then during deep inspiration a biopsy is taken.

We would now like to present our experience with six children in whom renal biopsies have been done:

RMM. An 8 year old Negro boy was first seen in August, 1955, for generalized edema. His growth and development and past medical experience has been entirely normal. Work-up in the hospital revealed a typical nephrotic syndrome; hypo-proteinemia, hypo-albuminemia, hyperlipemia, with edema and severe albuminuria. The urine did not contain any unusual formed elements. Blood pressure was normal. In the hospital he received several courses of steroid therapy over a three month period, at the end of which time he appeared clinically well, almost completely edema free, blood chemistries had reverted to normal and the urine was free of albumin. He was next seen in December, 1955, at which time he appeared well and was, therefore, put into a group of nephrotics that were being treated only with prophylactic Bicillin. He was next seen in January at which time he had a relapse. He gained 8½ pounds, and had become edematous 2 days before. His urine contained 4+ albumin. He was readmitted for more steroid therapy. During this hospital admission, he was again given several courses of steroid therapy but without too much success. He was sent home where attempts to treat with therapeutic doses of cortisone also were met with failure. He was finally readmitted to the hospital for the third time in April, 1956, and again his work up suggested a severe nephrotic syndrome. The renal biopsy was done at this time. Fig. 1-a reveals a photomicrograph showing two glomeruli which are stained with hematoxylin and eosin.

The glomeruli reveal some hypercellularity; a definite lobularity which is a reflection of basement membrane thickening; there is some proteinaceous material in Bowman's space which is diminished in size. The tubules contain protein material and their cells show some degree of degeneration. These findings are compatible with a diagnosis of the nephrotic syndrome. He remained in the hospital and never improved. He slowly went down hill, became almost anuric and died quietly in September, 1956.

F.B. was first seen when 2½ years old with the complaints of edema and elevated temperature. Physical examination revealed a bilateral hydrothorax, ascites, and generalized edema. The urine contained 4+ albumin, a few red blood cells and casts, specific gravity was 1.022, cholesterol was 464, total protein 3.3, albumin 0.4, globulin 2.9, blood pressure 108/82. This was in the pre-steroid era, so he was treated symptomatically and then follow-

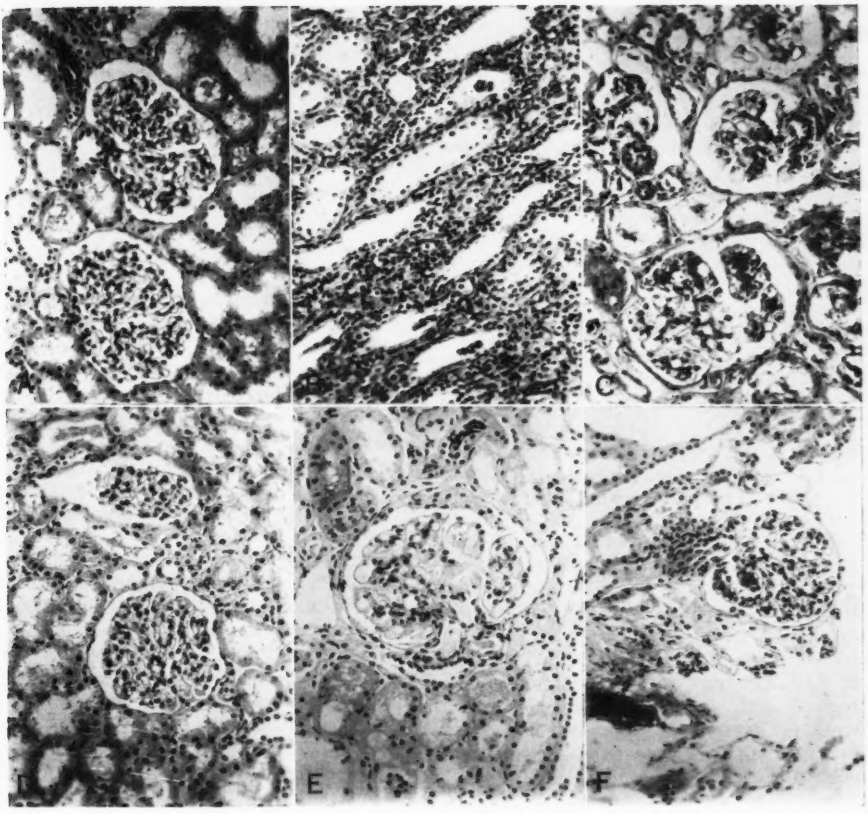

Figure 1

ed in the clinic on no specific therapy except a salt poor, high protein diet. He did fairly well, until 4 years of age when he had a relapse, was hospitalized again with the same general findings, but not quite as severe. At all clinic visits up to this time, his urine always contained some albumin. In 1953 hypertension was discovered. He went along this way until 1955 when his mother suddenly noted a left facial paralysis, which was confirmed at the clinic. Blood pressure at this time was 160/120, N.P.N. 43, total protein 7.7, cholesterol 168. Because of the hypertension and facial paralysis he was again hospitalized and this time the work-up revealed a fixed low specific gravity, mild azotemia, and severe hypertension. A renal biopsy was done. Fig. 1-B reveals a photomicrograph showing only hypertrophied and atrophied tubules. There were no glomeruli obtained at this biopsy. However, with the evident degree of tubular damage together with the clinical picture, one can project severe glomerular damage compatible with the diagnosis of chronic glomerulonephritis.

J.P., an eleven year old Negro boy was admitted to the hospital with a diagnosis of Henoch-Schoenlein Purpura with nephritis. This diagnosis was based on the history of sore throat, abdominal pain, joint pain and swelling, and a generalized urticarial purpuric rash. Laboratory tests revealed an A.S.O. titer of 625, mild albuminuria with many red blood cells and casts, beta-hemolytic streptococ-

cus was cultured from the throat. N.P.N. and blood pressure were normal throughout. He was treated with penicillin, bedrest and steroids. The steroids were used in the case of acute glomerulonephritis since the nephritis associated with Henoch-Schoenlein Purpura does not have the very excellent prognosis of ordinary acute glomerulonephritis. During convalescence, a biopsy was done. Fig. 1-C reveals a photomicrograph showing two glomeruli stained with hematoxylin and eosin and which reveal focal thickening of the basement membrane. There is some nuclear degeneration with some recent mild tubular epithelial damage. This is suggestive of the morphologic picture seen in lupus nephritis, but could also be the characteristic morphologic manifestation of the nephritis associated with the Henoch-Schoenlein syndrome.

H.B. was first seen when seven years old after the gradual development of generalized edema. He was admitted to the hospital where work-up revealed a typical nephrotic syndrome. Incidentally the cholesterol rose to 1472. He was in the hospital for two months and treated with A.C.T.H. The responded well; his weight dropped from 79 to 57 pounds. He was seen in the clinic several times, and his urine always contained 3 to 4 plus albumin, and many red blood cells. His blood pressure and NPN were never elevated. In October, 1953, he stopped coming to the clinic. In September, 1956 he was readmitted to the hospital at the age of eleven because of a sore throat, vomiting, and abdominal pain. Questioning the child and the mother regarding his health since 1953, brought out that he had been fairly well, and certainly had no obvious exacerbations. Laboratory tests revealed a mild albuminuria and elevated sedimentation rate. Beta-hemolytic streptococcus was cultured from the throat, the A.S.O. titer was 833, the total protein was 5.5, albumin 2.1, globulin 3.4, cholesterol 302. His streptococcal infection was treated with penicillin, but no specific treatment was directed toward the renal disease because it was assumed that he had a mild nephrotic syndrome. A biopsy was performed and the photomicrograph showed two glomerula stained with hematoxylin and eosin and mild changes in the renal tubules. The morphologic picture could very easily be interpreted as a normal glomerulus, however, the morphologic picture together with the clinical history suggest that this is the manifestation of a very mild nephrotic syndrome.

Y.W. is a twelve year old colored girl who was first seen in 1955. Her illness at that time consisted of fever, which fluctuated and did not respond to any antibiotics. She seemed pale and later proved to have a hemolytic process. Many other systems were involved: the joints, the blood vessels, the heart and also the kidney. The renal lesion was manifested by a mild albuminuria, a few red blood cells, some casts and hypertension. The diagnosis became quite obvious when a bilateral temporal alopecia and a quite typical erythematous rash was noted over the bridge of the nose, and on the face. At this time an L.E. phenomenon was demonstrated. She was placed on cortisone and the illness dramatically improved. The patient was discharged to the hematology clinic where the cortisone was gradually reduced. She got along fairly well for about eight months but recently, however, developed precordial pain, dyspnea, nausea, vomiting and fever and was readmitted to the hospital. Physical examination revealed some of the same findings as before, with the addition now of a pericardial friction rub, myocarditis, elevated sedimentation rate and severe hypertension. This time she was treated with intravenous A.C.T.H. and she gradually improved. During convalescence a biopsy was done. Fig. 1-E reveals a photomicrograph showing one glomerulus stained with hematoxylin and eosin. The glomerulus reveals patchy irregular and smudgy swelling of the basement membrane. There is a moderate degree of tubular damage with proteinaceous material in the tubules. These morphologic changes are compatible with early lupus nephritis.

T.H., an 8 year old colored boy, was admitted to the hospital for gradual development of generalized edema. Phy-

sical examination revealed puffiness of the face and pitting edema of the lower extremities, blood pressure was 100/65. Laboratory tests revealed persistent albuminuria, with no casts, red, or white cells, elevated sedimentation rate, N.P.N. of 70, cholesterol 387 mgs. per cent, total protein of 4.8, albumin 2.0, globulin 2.8. Because his illness appeared clinically mild the house staff wanted to try him no bedrest and no other treatment; however, after three weeks of no improvement, he was put on his first course of steroids with excellent results, in fact so impressed was the house officer that he stopped the steroids on the eighth day. Patient continued well for a while but eight days later again began putting on weight and the edema reappeared. A second course of steroids was then started and continued for ten days. Again he had an excellent clinical result. Shortly after this remission a biopsy was done. Fig. 1-F reveals a photomicrograph demonstating one glomerulus strained with hematoxylin and eosin. The glomerulus shows a marked degree of hypercellularity, moderate lobularity and basement membrane thickening. Bowman's space is markedly diminished. The tubules show a mild degree of degeneration. These findings together with the clinical story are compatible with the diagnosis of the nephotic syndrome.

It seems quite evident that from only six biopsies it is premature to make any statement about the correlation of clinical renal function with morphology in pediatrics. There is no doubt that after many more biopsies are done and correlative clinical and laboratory data are accumulated definite conclusions may be made. Percutaneous renal biopsy appears to be as safe a procedure in children as it has proven in adults, and it offers another valuable method for the study of renal disease in children.

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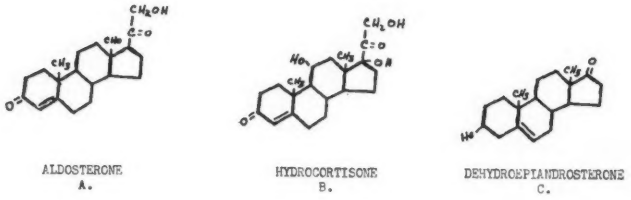
CIRCULATING PLASMA 17-HYDROXYCORTICOSTEROIDS — A MEASURE OF ADRENAL FUNCTION

J. J. GOLD, M.D.*



ALDOSTERONE
A.

HYDROCORTISONE
B.

DEHYDROEPIANDROSTERONE
C.

Figure 1

The adrenal cortex is becoming a progressively more complex endocrine structure, as our knowledge of its function enlarges. In spite of this increasing complexity however, each piece of knowledge helps to bring the total jigsaw puzzle nearer to completion. It is now generally accepted that the adrenal cortex elaborates in varying stages, steroids whose final functions fit into the following three categories:

1) Mineralocorticoids — Steroids in this category play a part in salt and water metabolism, and the physiologic adrenal product is aldosterone. (Fig. 1-A)

2) Glucocorticoids—Are substances concerned with gluconeogenesis, and are the steroids involved in the "permissive action" of adrenal hormones in stress. The physiologic adrenal product here is hydrocortisone. (Fig. 1-B)

3) Androgens—These may be represented by dehydroepiandrosterone. These substances exert anabolic and androgenic activity. (Fig. 1-C)

The glucocorticoids are felt to be the most important product of adrenal activity and it is the measurement of these substances in the blood (as a measure of adrenal function) with which this paper is concerned. It is important to note that hydrocortisone which contains a hydroxyl group at carbon 17, and similarly, reduced derivatives of hydrocortisone also containing hydroxyl groups at carbon 17 are the substances that are measured in the blood by methods currently available for clinical use. 17-desoxy steroids as corticosterone have to be measured by other methods.

Utilizing the Nelson and Samuels method[1] where normal values *for circulating plasma 17-hydroxycorticosteroids* are 4 to 10 micrograms percent, this same group[2] studied several patients with Addison's disease and no measurable blood corticoid was found. When ACTH was administered to normal patients there was an increase in the measurable blood corticoid to a peak of 20 micrograms percent. The peak was reached 60 minutes after ACTH was given intravenously with a return to the base line four hours later. When ACTH was given intramuscularly a peak was obtained in two hours and return to the base line was reached at six hours. When ACTH was given intravenously over a continuous period of time the increased levels were sustained and were higher than when ACTH was given in any other way. Normal blood levels of steroids were found in acutely and chronically ill patients, but in patients with terminal disease and who were terminal, the values were high. One must consider the effect of poor renal function as a contributory factor to these high levels. There were no measurable differences found in arterial or venous levels of cortical steroids. This same group[3] found a diurnal variation in the

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amount of blood corticoids found *with the highest amount* being present in the early morning. This tended to drop to a lower plateau over the day. Wallace, Christy, and Jailer[4], utilizing the Silber-Porter[5] method, found a range of 4 to 32 micrograms percent with an average of 16± 6.9 micrograms percent in normal individuals. Hospital patients with renal disease generally exhibited a normal range of corticosteroids in blood, but a few did have elevated levels. It is interesting that in this category of patients the urinary corticoids were low. In nine pregnant patients studied in the third trimester, values of 22 to 52 micrograms percent were obtained. Patients with Addison's disease had values of 0 to 21 micrograms percent; however two of these patients given ACTH exhibited no rise. Hypopituitary patients with secondary adrenal insufficiency had a range of 0 to 12 micrograms percent. Patients with Cushing's syndrome had values ranging from 29 to 107 micrograms percent. After bilateral subtotal adrenalectomy was done on these patients the values became normal. Patients with congenital adrenal hyperplasia had values of 0 to 20 micrograms percent. Perkoff and others[6] using the Nelson and Samuels method with a revised normal range of 13 ± 6 micrograms percent demonstrated that in diabetic coma the blood corticoids were elevated and when diabetic coma was overcome blood corticoid levels fell again within the normal range. Bayliss and Steinbeck[7] utilizing their method which has normal values of 3 to 16 micrograms percent administered ACTH to a series of patients and got a slight increase in plasma corticoids in 15 to 30 minutes, a definite increase in one hour and then this leveled off for the remainder of the infusion. Values up to 54 micrograms percent were obtained and when the infusion was stopped the level fell rapidly. When infusions of ACTH were repeated daily after six to eight hours a progressive rise in the steroid level was obtained suggesting that repeated daily stimulation increased the adrenal hormone output. When 80 units of ACTH gel was given in one injection to patients a significant rise of the steroid level up to 36 micrograms percent was obtained in two hours with a maximum concentration in four to eight hours. The level then usually fell off, with control values being obtained in 12 to 24 hours after injection. Bliss et al.[8] presented some data whereby they administered *one tenth international unit of ACTH* in a single intravenous injection and achieved a rise of blood corticoids from eighteen to twenty-seven micrograms percent in 15 minutes. By four hours there was a return to below control levels. Further study by Eik-nes et al.[9, 10], revealed that 25 international units of ACTH given intravenously over six hours caused maximal stimulation of the adrenals with a maximal steroid response in plasma. Addisonian patients showed a decreased response to this test even when control levels were normal and that has been the findings of other investigators. On the basis of these findings they suggested the use of intravenous ACTH as a test of adrenal cortical capacity in the human. When they administered ACTH to a patient with Cushing's syndrome due to adrenal adenoma they obtained a hyperactive response going from a fasting level of 35 micrograms percent to a level of 100 plus micrograms percent at the end of six hours. In various disease states such as hypertension, arthritis, etc., normal responses were obtained, as was the case in diabetes. Patients who had been maintained on cortisone for one to three months got a rise in plasma corticoids after ACTH in 36 hours in one patient and in 60 hours in another. Grumbach and others[11], did a study of plasma 17-hydroxycorticosteroids in patients with Cushing's syndrome due to bilateral adrenal hyperplasia using the Nelson and Samuels method. They found that many patients with Cushing's syndrome who had normal free circulating corticoids had increased conjugated circulating corticoids. The response that these patients had to ACTH was markedly hyperactive. Christy et al.[12] utilizing the Silber-Porter method found that normal patients went from control values of 4 to 23 micrograms percent to 35 to 54 micrograms percent after four hours of intra-

venous ACTH. Patients with primary Addison's disease showed no rise after ACTH even if they had normal control values. Patients with hypopituitarism having control values of 0 to 12 micrograms percent exhibited rises in plasma corticoids after ACTH but only a small percentage gave a rise as high as in the normal. Patients with Cushing's syndrome due to adrenal hyperplasia showed hyperactive responses to ACTH corroborating the findings of other investigators. After bilateral subtotal adrenalectomy in these patients, ACTH elicited a subnormal rise. Patients with congenital adrenal hyperplasia exhibited variable responses to ACTH, but most of them showed either no increase or only a slight increase in plasma corticoid. In patients with a normal pregnancy in the third trimester[13], control values were 32 to 46 micrograms percent and after ACTH they exhibited values of 79-111 micrograms percent which is a hyperreactive response. When patients with arthritis who were on Metacorten were given ACTH, there was a partial to complete prevention of the rise in plasma corticoids. When ACTH was given to patients with Cushing's syndrome due to adrenal carcinoma no rise in plasma corticoids was obtained. When ACTH was administered to a pregnant Addisonian patient no rise in plasma corticoid was obtained.

Bayliss et al[14], infused a total of 20 units of ACTH over an eight hour period to a 41 year old patient with the adrenogenital syndrome. A rise in 17-ketosteroids was obtained, but no appreciable rise in plasma corticoids above the control level of three micrograms percent was found. Eik-nes and others[15] administered hydrocortisone and cortisone intravenously to some normal patients and found that the rate of disappearance of these compounds from the plasma was related directly to the plasma concentration of the steroids. A peak was obtained in one hour with a return to control levels in six hours. The clearance from the plasma of orally administered steroids was variable, with that given by microsuspension being quicker than tablets. A peak was obtained in one hour with a return to control levels in eight hours. When the material was given intramuscularly a peak was obtained in two hours and control levels had not been reached at the end of twelve hours.

Further studies on the changes of plasma 17-hydroxycorticosteroids have involved their examination in surgical states as well as in liver disease. Franksson and Gemzell[16] did such studies in the preoperative period. They found a rise in blood corticoids from control levels two days before surgery at 5.53 micrograms percent to 14.51 micrograms percent on the day of surgery and the authors felt that this rise was due to psychogenic stress. Sandberg et al[17] in a similar study on the effects of surgery on the plasma corticoids found that the plasma corticosteroids were elevated following the induction of anesthesia, and during and after surgery. The elevations in plasma corticosteroids following surgery were roughly proportional to the duration and severity of the operative procedure. It was noted that the elevation usually persisted for at least six hours following the surgical intervention. In evaluating the effects of infused ACTH on these same patients the authors administered ACTH both preoperatively and again post-operatively. Even when the post-operative levels of 17-hydroxycorticosteroids rose spontaneously to levels as high as those induced in normals by ACTH, the infusion of ACTH post-operatively under these conditions led to an additional increment. When the metabolism of intravenous hydrocortisone was compared pre-operatively to post-operatively, it was noted that the post-operative patients did not metabolize the infused hydrocortisone as rapidly as they did prior to surgery. The authors concluded that apparently the adrenal is submaximally stimulated by surgical trauma but impaired disappearance of the circulating steroid also contributed to the rises observed. The same group of authors[18] further pursued the impaired metabolism of plasma corticosteroids by doing concomitant BSP excretion studies. It was shown that the post-operative rises in plasma corticosteroids showed a direct correlation with

impaired BSP excretion. The conjugated urinary 17-hydroxycorticosteroids rose after all except one brief minor procedure but showed no correlation with the plasma levels attained. Their conclusions were that the increased plasma level of 17-hydroxycorticosteroids after surgery was the result of both increased adrenal secretion of these steroids as well as impaired hepatic removal.

Klein et al[19] measured both free and conjugated plasma corticosteroids utilizing the method of Nelson and Samuels. This study was specifically gauged to determine any difference between the serum corticoids in normals and in those patients with liver disease. With their method the average normal control serum corticoid was 13 gamma percent, the average normal control conjugated serum corticoid was 8.5 gamma percent. In adult cirrhotics the average free plasma corticoid was 10 gamma percent and the conjugated 1.7 gamma percent. The authors then administered cortisone acetate orally to normal individuals and obtained a rise in conjugated serum corticods that followed the rise in the free corticoid levels. The elevation of the conjugated corticoids persisted longer with the peak occurring later than that obtained with free corticoid. However, two to three hours after the cortisone acetate had been administered to cirrhotic patients, the free corticoid level roughly followed that found in the normal individual except that the peak persisted about an hour longer. The conjugated fractions started at low levels and showed only a minimal rise. When ACTH was administered to normal individuals there was a prompt rise in both the free and conjugated corticoid levels. The same amount of ACTH to patients with liver disease demonstrated a normal rise in free corticoids but none to a slight rise in a conjugated fraction. The authors concluded that the patients with advanced liver disease conjugate exogenous or endogenous corticoids slowly and that the rate of removal is slower than normal. Brown et al[20] infused hydrocortisone and tetrahydrocortisone to a series of normal patients and to another series of patients with liver disease. When hydrocortisone was infused to both normal patients and patients with liver disease there was an initial rapid disappearance of the 17-hydroxycorticosteroids from the plasma. One hour after the start of the infusion only about 5 percent of the infused steroid remained. Then came a period lasting many hours where the plasma 17-hydroxycorticosteroids disappeared at a slower rate. The rate here was inversely proportional to the degree of liver damage as measured by BSP. When tetrahydrocortisone was infused it was noted to disappear at a much more rapid rate which was independent of liver function. It was noted that patients with liver disease had normal fasting levels of plasma 17-hydroxycorticosteroids, but excreted less in the urine than did the normals.

Plasma corticosteroid studies in pregnant women and in normal children and in children with various diseases have been done by many investigators. Bayliss et al[21] recently reported some studies on a series of pregnant patients. They measured the plasma corticoids at monthly intervals in 30 pregnant women, and noted that after approximately the third month of gestation a progressive rise in plasma corticosteroids was apparent, and this continued to term. After parturition the level fell to normal within one week post-partum. The same authors measured corticoids in umbilical blood and found these to be elevated. Robinson et al[22] in a similar study found that plasma corticosteroids rose in pregnancy to a mean of about 33 gamma percent. They stated that the level was of the same order of magnitude in both the early and late stages of pregnancy. In contradistinction to the findings of Bayliss these authors found that it generally took some six to nine weeks for the plasma corticosteroid levels to return to normal post-partum levels, though in some patients this did occur in a few days. Apparently this finding is unique with the Robinson group because Gemzell and others also reported a return to normal levels within six days. In a very interesting study, Gemzell[23] found a maximum of 20 gamma percent in pregnant women in the last trimester. However, with cesarian section the moth-

ers' plasma corticosteroids were essentially as just mentioned but the infant had a plasma level of only 2.9 gamma percent. Carrying this a little further the authors studied the plasma corticosteroids in primiparous patients as well as in their infants. In this case a mean of 42 gamma percent was found for the mother and 28 gamma percent for the infant. A similar study on multiparous patients revealed a plasma corticosteroid of 23.4 gamma percent in the mother and 15.8 gamma percent in the infant.

Klein et al[24] measured plasma corticosteroids in a series of 14 newborn infants two to five days of age. It was noted that their blood corticoids were only 0.9 gamma percent. Normal infants ranging in age from 3 weeks on had levels of 13 gamma percent as found in the adult. The authors hypothesized from this that apparently the material hormonal levels depress the fetal pituitary and the low infant levels for the first week of life are due to depressed pituitary function. Then the infant's pituitary-adrenal axis begins to function normally and the blood corticoid levels rise to their expected values. Carrying this one step further the authors in another study[25] then administered ACTH to children, and three day old infants. In the infants the control values averaged 2 gamma percent. One hour after ACTH infusion the level rose to 26 gamma percent and two hours after ACTH to 45 gamma percent. In normal children, the mean control level was 14 gamma percent, one hour after ACTH rose to 33 gamma percent, and two hours after ACTH was 31 gamma percent. Control levels on one infant averaged 0.5 gamma percent. This infant was then subjected to operative trauma and post-operatively the blood level rose to 100 gamma percent. The authors concluded from this study that though the low levels of plasma corticosteroid in the newborn suggest adrenal hypofunction probably due to suppression of the fetal pituitary by maternal hormone levels, this hypofunctioning adrenal responded readily to stress and ACTH stimulation.

Ely, et al[26-29] in a series of papers measured plasma 17-hydroxycorticosteroids in children. They found that there was no significant difference in levels found in children with various diseases as compared to normal children. The only instance in which low levels of plasma 17-hydroxycorticosteroids were found was in cases of the adreno-genital syndrome. In normal children administration of ACTH caused a prompt but trancient elevation. They then measured blood levels of 17-hydroxycorticosteroids in children with acute rheumatic fever. They found that children having acute rheumatic fever for two weeks or more had blood corticoid levels of 5.9 gamma percent. Their normal mean value was 12 gamma percent. Low values were also found in inactive rheumatic fever. ACTH levels were found to be elevated in these groups. When exogenous ACTH was given they got a normal rise in blood corticoid. During the first week of acute rheumatic fever, levels of 23.1 gamma percent were obtained as well as low ACTH levels. From this study the authors concluded that patients with acute rheumatic fever have a relative adrenal insufficiency that responds to ACTH. It must be noted that this conclusion is not a generally accepted one.

Summary

The clinical application of the measurement of plasma 17-hydroxycorticosteroids in varying states as well as the response of these steroids to ACTH stimulation has been presented. The importance of the latter response has been stressed as a means of evaluation of adrenal function.

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CLINICOPATHOLOGIC CONFERENCE

DR. H. J. ISAACS, Chairman **DR. H. RAPPAPORT, Secretary**

Presented at Mount Sinai Hospital, Chicago, Illinois

L.N. No. 259340. The patient, a 23 year old white female, gravida IV, para II, was admitted to Mount Sinai Hospital—Gynecology Department—on April 25, 1955.. The patient appeared to be pale and physically exhausted and gave a history of prolonged and heavy menstrual period of a seventeen days' duration. On admission the temperature was 99°, pulse 72 and regular, respiration 18 and blood pressure 110/70.

Gyne Physical Examination on Admission: Multiparous introitus, B.S.V.—negative. No cystocele or rectocele. The cervix pointed posteriorly and was freely movable. Corpus anteflexed, multiparous sized adnexa and cul-de-sac essentially negative. The abdomen was soft, and no masses were palpated. The breasts were small and contained collostrum.

Laboratory Data on Admission: *Urine analysis*: pH 7.5; Sp.Gr. 1.005; Protein —1+; sediment initially contained only 50-75 WBC and numerous bacteria. Subsequently, two days later, the sediment showed the presence of 12-15 RBC; 12-15 WBC and occasional Epithelial cells. No casts. *Hemogram*: Hgb. 9.2 (59%); RBC 3.3 M; C.I. 0.89; WBC-5,250; Segs-56; Eos-2; Bas-1; Sm. Lymphs-37; Mono-4. Platelets 168,000. Bleeding time—1 min. Prothrombine time—15 sec. Control 13 sec. and clotting activity 73%. Blood Kahn, Kline and Wasserman tests were negative. Biochemical data: 4/26/55: Serum glucose—121; BUN—78.5. Two days later serum glucose was 128; BUN 124; Creat. —12.2.

The original diagnostic impression was *INCOMPLETE ABORTION WITH FUNCTIONAL BLEEDING* rule out blood dyscrasia. On April 29, 1955, a D & C and biopsy of the cervix under general anesthesia was performed. Gross and microscopic examination of the specimens showed placental tissue—chronic cervicitis and epidermidization. The uterine pack was removed on April 30, 1955. A small amount of vaginal bleeding occurred after removal. At that time, the patient was profoundly anemic; the eyelids were swollen and the patient complained of nausea. Laboratory data revealed an *increasing azotemia* and it was felt that the patient should be transferred immediately to Medical Service for a complete work-up.

Upon transfer to the Medical Service on 5/1/55, a review of the patient's history and findings read as follows: 23 year old married female with one year's history of menorrhagia with periods which lasted up to ten days on several occasions, the last one lasting 17 days. Also, the patient complained of easy fatiguability for the past twelve months, which had become worse during the past three weeks, and a ten pound weight loss during the last four months. The patient also noted bilateral ankle edema at the end of each day. Her appetite was fair and B.M. regular.

Past History: The patient was born in Belgium and had been in the U.S.A. for two years prior to admission. At the age of five, she had a "red generalized rash" all over the face and body. For as long as she could member, she always had polyuria and nocturia (3-4x) but no polydypsia. At the age of 16, she spent twelve days in bed because of "an infection in the throat." She denied epistaxis and joint pains. Menses at the age of 13, periods regular, every 28 days prior to one year ago and accompanied frequently by cramp-like pains in the lower abdomen. She also stated that she was very nauseated through all of her pregnancies. Her oldest child is a four year old boy. She also has a three year old girl. She had one abortion just prior to this admission. In November of 1954, the patient was hospitalized at C.C.H. where a retrograde pyelogram was taken. She was told at that time that she had "kidney trouble" and received a course of penicillin therapy.

LABORATORY DATA

Bl.Cts.:	RBC	Hgb.	WBC	Segs	Stabs	Eos	Bas	Lym.	Monc	Platelets	C.I.
5/1/55	3.0	9.0	4,550	56		4	3	32	7	208,000	0.97
5/4/55	3.5	11.4	5,150	63	2	7	-	19	8	-	1.04
5/8/55	4.3	12.7	-	-	-	-	-	-	-	-	0.94
5/14/55	3.6	9.8	14,150	82	1	2	1	11	2	-	0.86
5/20/55	3.6	9.8	-	-	-	-	-	-	-	-	0.87
5/28/55	3.4	10.1	12,400	85	3	-	-	9	3	-	0.93
6/4/55	2.6	8.4	10,500	83	2	1	-	11	3		1.03
6/14/55	3.0	9.2	-	-	-	-	-	-	-	-	0.98
6/20/55	3.5	10.4	11,200	89	5	-	-	4	2	228,000	0.96

Bl.Chem.:	Gl.	Urea N.	Creat.	Cl.	Na.	K.	pH.	CO	P	Ca.T&I.	T.P.	Alb.	Glob.
4/30/55	95	101.5	-	101	130	4.5	7.25	9.2	-	-	-	-	-
5/2/55	-	99.4	12.6	107	141	4.6	7.21	18.5	-	-	6.9 A/G-106	3.55	3.35
5/5/55	-	86.8	-	104	144	4.4	7.31	23	-	3.75-1.68	-	-	-
5/7/55	-	97.3	11	99	129	5.0	7.35	14.4	-	-	-	-	-
5/12/55	-	195.8	11.2	97	130	4.4	7.25	15.3	-	-	-	-	-
5/14/55	-	106.4	12	94	135	4.7	7.27	18.5	-	-	-	-	-
5/18/55	-	100.1	12	89	125	4.9	7.31	18.5	5.5	4.7-2.3			
5/23/55	-	99.4	14	89	126	6.2	7.19	12.1	-	-	-	-	-
5/28/55	-	84.8	13.3	96	125	4.1	7.3	12.2	-	-	-	-	-
6/3/55	-	89.99	16.2	99	133	3.3	7.18	13.5					
6/10/55	-	99.5	17.6	96	133	4.5	-	-					
6/20/55	-	-	-	89	130	5.4	7.25	-					
6/23/55	-	160	22	85	135	5.6	7.4	13	-	- A/G-1.07	7.35	3.8	3.55

Urinalyses:	pH	Sp.Gr.	Pro.	Sug.	Acet.	Casts	RBC	WBC	Epith.	Bact.
4/30/55	6.5	-	1+	0	0	0	0	occ.	occ.	many
5/6/55	-	-	-	-	-	-	2	1-3	occ.	-
5/10/55	7.0	1.007	2+	0	0	0	12-15	3-5	few	few
5/17/55	7.0	1.010	4+	0	tr.	0	28-30	3-5	mod.	-
5/24/55	5.5	1.005	1+	0	0	0	many	-	-	-
5/31/55	5.5	1.005	1+	0	0	0	occ.	20-25	occ.	-
6/11/55	5.5	1.008	0	0	0	0	occ.	150-200	few	num.
6/21/55	5.5	-	4+	0	0	0	20-25	many	many	
6/29/55	5.5	1.010	4+	0	0	0	-	many	few	many

Urine culture: 4/30/55 - E. Coli isolated.
5/5/55 - Enterococci & A. Aerogenes.
6/3/55 - Hemolytic Staph. aureus.

Addis Count: 5/1: RBC 749,995 WBC 3,596,940 Casts - 0

L.E. Cells: 5/1/55: Neg. in peripheral blood.
5/31/55: Neg. in peripheral blood.
6/28/55: Neg. in peripheral blood.
5/14/55: Neg. in bone marrow.

Thorn Test: 5/2/55: 9:30 A.M. 139/cmm
2:30 P.M. 49/cmm

5/2/55 - Serum Bilirubin - Total 0.1; direct .05, Cholesterol 215, Esters 64%, Thymol turbidity 2.1, Cephalin-Cholesterol flocc. test - neg.

LABORATORY DATA (continued)

5/14/55 - Bilirubin 0.2; direct 0.05.
5/2/55 - Serum Alpha Globulin 0.75, Beta 1.76, Gamma 0.84
5/2/55 - C-Reactive protein - 1+
Antistreptolysin Titer - 50 Todd u.
5/5/55 - Red Cell mechanical fragility test:
Pt. 6.14% Control 3.2% Normal 1-7%
5/6/55 - 24 h. Urine for Urobilinogen:
Bilirubin - neg.
Urobilinogen - 7 E.U.
5/16/55 - Urobilinogen - 9 E.U.
5/30/55 - Coomb's test, direct and indirect: Negative.

Examination of feces: 5/6/55 - Bilirubin - 1+
Urobilinogen - s. positive.
5/10/55 - Bilirubin - 2+, Urobilinogen-moderately positive.
5/9/55 - Bilirubin-trace; Urobilinogen-mod. pos.

Repeated urine sputum cultures for T.B. were reported negative.

5/3/55: Bone marrow: No diagnostic abnormalities.

E.K.G.: 5/5/55 - Within normal limits.
5/14/55 - Possible hypopotassemia.
6/7/55 - Possible hypopotassemia.
6/29/55 - Rate 95; nodal rhythm; no P waves; T_3 inverted; T_1 biphasic. Compatible with posterior infarct.

X-rays: 5/3/55: Chest: normal.
Abdomen: 1) Gaseous distention of small bowel loops suggesting adynamic ileus or early intestinal obstruction.
2) Mass in pelvis, which could be produced by the distended urinary bladder or uterus.

5/14/55: Chest: Enlarged heart, together with pulmonary congestion of a type that might occur with uremia.

5/17/55: Chest: Findings as on previous film.

6/14/55: Chest: No bony pathology.
1) Minor amount of left pleural fluid noted.
2) Heary markings are noted in the lung field on both sides. There is no definite gross infiltration to suggest pneumonic consolidation. However, a minor peribronchial infiltration of broncho-pneumonic type in the right base is not excluded.
3) The heart size is at the upper limits of normal.

6/26/55: Chest: Left sided pleuritic effusion. Bronchopneumonic infiltrations in the left mid-lung field and the right lower mid-lung field.

Physical Examination: B.P. 130/82; pulse 84/min. and regular. Respiration—18. Patient in supine position, in mild respiratory distress, moderately dehydrated; skin dry; eye balls soft; funduscopic examination—negative; brownish-pigmentation involving the malar regions and the bridge of the nose was noted (Butterfly distribution). As far as the patient could recall, she had had this pigmentation since childhood. She added that this condition was aggravated, and extended to the lips and eyebrows under direct sunlight. She was certain that this pigmentation was present long before her first pregnancy. She had not noticed any significant change in its character in the past few years.

The chest was symmetrical, both sides expanded equally. The breasts were small and no masses were palpated. The lungs were clear to P & A. The heart was within normal limits to percussion. A Grade II systolic murmur was audible over all valve areas but was loudest at the apex. The abdomen was flat and soft. No viscera or masses were palpated. There was slight tenderness in the right and left lower quadrant on palpation. Rectal examination was essentially negative. The sensorium was clear. Superficial and deep reflexes were physiological and there were no pathological reflexes. Extremities were edema free.

Hospital Course: Upon transfer to the Medical Service, the patient was put on complete bedrest and intravenous fluids including 5% glucose in water and lactate solutions were given to correct the electrolyte imbalance and the acidosis. The intake and output of fluids were satisfactory, ranging at the beginning from 2,000 to 3,000cc of urine daily. The blood pressure ranged between 150-110 systolic and 95-70 diastolic. Occasional nausea and vomiting occurred. During the second week of hospitalization, the patient developed a U.R.I., nasal congestion and a temperature of 100°. Terramycin was given and at the same time she was receiving multivitamins and dramamine for the relief of the nausea.

On May 6, 1956, vaginal bleeding was noted and interpreted by the Gynecologist as due to endometritis. Erythromycin was added to previous therapy. The vaginal bleeding lasted for about 10 days and decreased gradually. Spotting persisted for a much longer time. On May 11, 1955 (11th hospital day), a protodiastolic *gallop rhythm* was heard. The following day, the patient complained of a slight chest pain. Due to the patient's intolerance for food, the diet was changed rather frequently and she was intermittently on regular, soft, bland and medical liquid diets. On May 13, 1955, the total intake (including 2,000cc. of intravenous infusion) was 2,900cc. and the urinary output was 2,090cc.

Shortly after midnight on May 14, 1955, the patient began to cough and expectorate bloody sputum. Respiration became dyspneic and the patient complained of slight chest pain. B.P. was 140/90, pulse 140 and regular and temperature 102°. The skin was warm. Percussion revealed a decreased resonance over the right lower lobe. Coarse rales and rhonchi were audible over both bases. Gallop rhythm was present. The extremities were edema free. The impression was bronchopneumonia with pulmonary infarction. In addition to previous therapy, the patient was placed in an oxygen tent and digitalized with intraveous Cedilanid and later, put on maintenance dose of Digitoxin. The urinary output remained good, (1,900cc).

Following this episode, the nausea and vomiting increased. Thorazine was started. Re-examination of the eyegrounds on May 17, 1955 (24th hospital day) showed slight diffuse edema and blurred disks. Two days later, a friction rub (systolic) in the 4th interspace just left to the sternum was noted. At the same time, a progressively increasing edema of the presacral region, thighs and ankles was noted. The patient's condition was becoming worse; nausea and vomiting were almost constant, the food intake was very poor, occasional muscle twitchings were seen and the urinary output was only 500cc. Bloody discharge from the vagina was still present. In addition to the previous therapy, on May 22, 1955, the patient was given 20 Units of ACTH daily. A few days later, some improvement

was noted and appetite increased. The patient felt stronger and the urinary output increased. The face was puffy, but the peripheral edema cleared up.

Since the time of the patient's hospitalization there had been a persistent low-grade type of anemia for which she received transfusions of whole blood or packed cells.

However, on June 10, 1955, a sudden drop of RBC and Hgb. from 9.8gm. to 6gm. was noted. The possibility of Chloromycetin toxity was considered. On June 20th, numerous petechiae developed over the anterior surface of the thighs, and a chilling sensation was noted. The temperature remained normal. The patient's condition was reported as "getting worse." Nausea and vomiting were continuous. Her face was puffy and the ankle edema increasing. Gallop rhythm persisted. On June 26, 1955, the ACTH was discontinued and replaced by Meticorten. However, the peripheral edema increased and the condition of the patient remained serious. On June 28, 1955, Meticorten was discontinued and ACTH re-instituted. At that time, besides ACTH, the patient received Testosterone—25mg. twice weekly, Chloromycetin—1Gm. (I. M.) B.I.D., Penicillin—400,000 U. daily, Basogel—30cc. Q.I.D., Sodomytal—gr. 1 q4h, Elixir of Donatal, Thorazine, Digitoxin, vitamins and intravenous fluids.

On June 28, 1955, the patient developed pulmonary edema with bloody expectoration, dyspnea, pulse 140/min., B.P. 140/100. Oxygen was given by positive pressure mask and Cedilanid injected intravenously. Dullness was noted over the left base and 100cc. of clear fluid was removed by thoracentesis. On the following day the patient's condition became worse, bloody expectoration and moist rales over the pulmonary fields persisted. The patient spent the night in the oxygen tent. Temperature was 99°; pulse 80/min, regular; B.P. 160/80; respiration 32. The heart tones were obscured by moist rales. The liver was palpated 3-4 fingers below the right costal arch. 2+ edema of the lower extremities and edema of the vulva was noted. The urinary output during these last two days was about 800cc. each day. Oral intake was practically zero because of vomiting. On June 30, 1955, the patient appeared edematous, acutely ill, in pulmonary edema and expired at 9:30 P.M. (67th hospital day).

The laboratory data preceding death reads as follows: Serum Urea N, 193.2; Creatinine, 28; Cl, 90.6; Na, 126.7; K, 7.71; Ca 3.45, total and ionized Ca, 1.7; CO_2, capacity, 11.5; Serum pH 7.27. RBC, 2.5M; Hgb. 7.58 (48%), C/I .94. Urine pH. 5.5; Sp. Gr. 1.010; Proteinuria 4+; many WBC and a few epithelial cells reported in the sediment.

During the hospitalization, the patient's weight ranged between 108-116 lb.

Dr. Kadin*: In the evolution of acute glomerulonephritis, we know that anywhere from 10 to 40% of all acute glomerulonephritis cases progress to chronic glomerulonephritis. But, in the evolution of chronic glomerulonephritis, quite frequently in these cases involving a female, we have a phase of nephrotic syndrome which she did not have.

We have a woman who had no evidence of a toxic type of kidney due to such chemical poisons as carbon tetrachloride, mercury, arsenic, etc. We have an apical systolic murmur which poses the question whether this could be a case of subacute bacterial endocarditis with embolic phenomena to the kidney causing an infectious nephritis. However, embolic phenomena of such a degree would also appear elsewhere in the body. Also, from what I have read and I have seen, embolic phenomena are not of such degree as to cause uremia, although they can have acute glomerulonephritis along with the subacute bacterial endocarditis. I do believe that this played a role in this particular case.

The possibility of collagen disease has to be considered, e.g. polyarteritis, lupus erythematosus, and other so-called collagenous diseases. The L.E. phenomena was negative four times in this girl. If

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L.E. cells are looked for, it is reported that almost invariably they are found in lupus erythemantosus.

The question of whether the pigmentation on the patient's face was due to collagen disease arises. Three dermatologists saw her, and it was agreed that the pigmentation was not of the collagen type.

The course of this disease in a woman asymptomatic, relatively speaking as far as her renal status is concerned, going along relatively well and then having an acute terminal course, with the typical factors of uremia, pneumonia, infarction, cardiac failure, possible fibrinous type of pericarditis, and acute death with right heart failure, is rather a typical story of uremia due to renal disease.

The question that arises is what is the renal disease? From what I have been able to discern, I would say that the possibility of a chronic polycystic kidney must be considered. She had no hematuria of any large consequence, nor did she have a history of overt blood in her urine, which quite commonly occurs in polycystic disease. Yet, she was told that she had "kidney trouble." There is no family history of polycystic disease. Although family history in this case is negligible, she would have had some type of clue, possibly in her famital background, giving polycystic disease as an etiologic factor. If this were so, other anamolies associated with polycystic disease would have to be considered.

The possibility of chronic glomerulonephritis must be considered. It could have been a factor in the two spontaneous abortions. However, in the history, the anemia was not marked, the test was relatively normal. The absence of nephrotic syndrome and the general pattern, to me, makes this possible, yet not most likely. The story of four repeated pregnancies, with two spontaneous abortions in the last two pregnancies, is a very good cause for chronic pyelonephritis. Asymptomatic pyelonephritis is very common, and in a woman who possibly did not have the best of medical care, the occurrence of polyuria with a pregnancy must be assumed by her to have been due to pressure on the bladder, and possibly this chronic pyelonephritis in her first two pregnancies was passed by; the quality of her prenatal care during her third pregnancy is even questionable.

The story of a woman going along relatively asymptomatic with chronic uremia and then having a sudden downcourse and demise, with terminal hypertension, makes me believe that this was a chronic pyelonephritis with all its attendant renal disease. Whether the pathology department can differentiate chronic pyelonephritis from chronic glomerulonephritis is always a good point. I believe that this woman had a chronic pyelonephritis with possibly a destroyed kidney and perhaps a chronic glomerulonephritis as a prefix.

I would like to have the x-ray department discuss the patient's x-rays; also, if Dr. Isaacs would discuss the electrocardiograms in regard to the unique changes that occurred with the marked electrolyte imbalance I believe this could be of great value.

Dr Arendt*: It is unfortunate that during the entire period of observation and treatment, no roentgenological examination of the kidney was ever ordered here. The diagnosis of pyelitis could well have been made, or at least supported, by an x-ray film showing a dilated kidney pelvis. The functioning of the kidney, its Diodrast clearance, might have given a valuable point. The configuration of the kidney and calyces, and the outline of the ureter, might have been important, but no films were available.

The flat film of the abdomen shows a density filling the lower abdomen which suggests either an enlarged uterus or bladder, very much distended. No fetal parts are visible and it is impossible, without contrast media, to differentiate between a distended bladder and an enlarged uterus. A film taken after emptying the bladder would clear this point. The kidney region on the film is com-

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pletely covered by distended small bowel loops which do not have the characteristic pattern of ileus, either mechanical or paralytic, but are meteorismotic, not rarely seen with kidney pathology, stones as well as infection.

The chest films, all taken as portable films, have the limitations of portable films. There is considerable enlargement of the left heart and a lung picture with changing, spotty infiltrations, suggestive of developing bronchopneumonia and, in later stages, what we thought was lung edema.

Dr. Isaacs*: The electrocardiograms were taken between the dates of May 4, 1955 and June 29, 1955. The first cardiogram taken shows a high peaked T-wave with a prolongation of the QT interval. This makes you feel that there is some electrolyitic disturbance such as hyperpotassemia. The second electrocardiogram was taken on the fourteenth, ten days later, and there is a marked change in the T wave. Now there is a decrease in the voltage of the T wave in lead 1 and 2, with some prolongation of the QT interval, which makes you feel that now there are findings of a hypopotassemia.

The electrocardiogram which was taken three days later showed more distinct changes in that the T wave in the 1st and 2nd leads, which on the first examination showed a high peaked T wave, and on the second examination a very low upright T_1 and T_2, now shows T waves becoming inverted in the first and second lead and inverted in all the V leads: V1, 2, 4, 6 and *A,V,F*. Therefore, if our conclusion is correct, this would speak more for electrolytic disturbance most likely on a basis of a hypopotassemia and possibly a decrease in the calcium as well.

The next cardiogram was taken five days later, in the month of May, and shows more deeply inverted waxing T waves in the V leads. This would make us feel that possibly there was a much lower potassium and calcium content. The fifth EKG showed the T waves waning. Three weeks after that, another cardiogram was taken, showing that the T waves were upright in the first lead and not so inverted in the second or third leds and in a V lead.

Our conclusion, if correct from a viewpoint of interpretation of the electrocardiograms, is that the first EKG was suspicious of a hyperpotassemia and that the subsequent EKG showed signs of a hypopotassemia and hypocalcemia. Some evidences of a left heart strain were also present.

Dr. Kadin: In reading the hospital course, it is interesting to follow the results of therapy administered for the uremic state in that, no matter what is done to treat the electrolyte imbalance it will still vary considerably. I think what it does tend to bring out is that in the uremic state one should not be vigorous with too much therapy because of the narrow balance of electrolyte and fluid in the body. Despite the so-called adequate output, these people do accumulate fluid and go into failure. My final diagnosis is chronic pyelonephritis, possibly a chronic glomerulonephritis of a low degree.

Dr. Isaacs: Without knowing anything about the patient, either history or findings, sometimes it is very difficult to differentiate electrolyte imbalance with changes in potassium and changes in the T waves, such as hypopotassemia or other really cardiac changes reflecting heart involvment, chronic coronary disease, and others. However, that is the chance one has to take in reading electrocardiograms by itself without consultation with the internist and without consultation with the history and findings *per se*.

I admit that the ideal way of interpreting electrocardiograms, and the same thing applies, I am sure, to x-ray, is to know all about the clinical story of that given patient, both in regard to history, findings and laboratory, for only then can

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adequate interpretations be made. For instance, we know electrolytic disturbances are seen with the uremic state. Since the chart described a uremic state, I think at least one interpreter of electrocardiograms would interpret that as being due to electrolytic disturbance.

Dr. Einhorn*: I was going to extensively discuss the rash, but I find that this is now unnecessary. When the patient was in the hospital, the rash aroused great controversy and, as I recall, one dermatologist had said that it was not compatible with L.E. Another dermatologist said that it could be due to L.E. According to the abstract, she was positively certain that this pigmentation was present long before her first pregnancy, but actually, after the patient was in the hospital for a period of time, it was obvious that she could not tell us definitely when she first got the pigmentation.

In addition, she was in the hospital a month before we discovered that she had previously been in County Hospital. A phone call to County Hospital disclosed the following: a retrograde pyleogram has been done and this revealed a deformity of the upper and lower calyx of the right kidney, absence of the middle calyx, and irregularity of the lower third of the right ureter. The left kidney showed loss of normal cupping of the calyces and marked narrowing of the infundibulum. All of the changes described were considered compatible with a chronic inflammatory process.

Dr. Levine**: There were fundoscopic findings on this patient which were completely negative. The patient had a normal blood pressure throughout, clear to the end. A pyelonephritis can cause severe kidney damage without elevation of pressure. But when the terminal phase of pyelonephritis is reached, often a very high blood pressure is seen. Some of the highest blood pressures at terminal stages of kidney failure have been with what we have seen here — with pyelonephritis. This patient went into heart failure without any elevation in blood pressure.

I think, even for the sake of discussion alone, rather than just to make a diagnosis, there are other possibilities that should be mentioned.

Did this patient have, for example, an amyloid condition? I know these are rare conditions but I also think they should be brought cut, too; they are possibilities. We know that kidney damage, proteinuria, and other types of kidney disturbances, are found and can go on to an end stage without any elevation in blood pressure.

They may have severe anemia, so that we might explain the anemia here. The patient had been hemorrhaging. And, they do have heart failure as well and they need not necessarily have the major manifestations that we ordinarily look for, as skin changes and macroglossia, or any of the findings we used to be told are necessary for the diagnosis of an amyloid kidney.

And there is another thing I think should be brought up. I think we should still say something about the possibility of a subacute bacterial endocarditis. I do not think that there were any blood cultures done. The patient developed petechia and she also had a murmur. You may see petechia in uremia and have a systolic murmur due to anemia; but I do think you can have kidney changes with subacute bacterial endocarditis that are more than just a focal glomerulitis, and which may go on to more severe renal damage than is ordinarily looked for.

Dr. Isaacs: We have a record of the blood pressure being 140/100 and I think that's somewhat elevated, at least the diastolic pressure, and following that 160/80. Of course, when you analyze a pyelonephritis you find that it's much more common in females, that they may have one or more episodes of pyelitis, but they make an uneventful recovery and may be symptom free for 5, 10 or 15 years, and then suddenly may develop

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a chronic uremic picture with heart failure, etc. When we have a female with a story like this, who's gone along for many years symptom free, at least you think of the possibility of a pyelonephritis for this is a sequence of events which can occur quite frequently in females.

Dr. Rappaport: Post mortem examination revealed a young, well developed, and apparently well nourished white female with the main pathologic findings in the genitourinary tract. Both kidneys were considerably reduced in size. The left kidney weighed 57 grams; its capsule was thickened and stripped with great difficulty, leaving a coarsely nodular surface (Fig. 1.) On section the demarcation

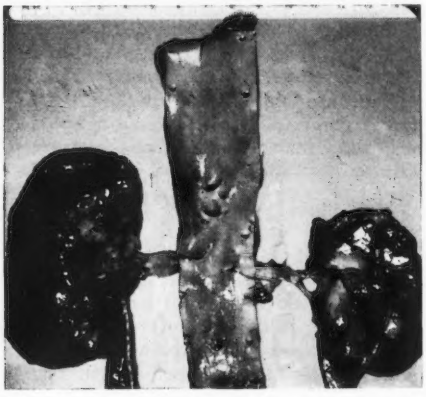

FIGURE 1

Bilateral pyelonephritis. Posterior aspect of kidneys; both are reduced in size, the right more so than the left. Note the small caliber of the right renal artery.

between cortex and medulla was obscured and there were irregular areas and strands of yellowish-grey tissue replacing the normal kidney parenchyma. Cortex and medulla were considerably reduced in thickness, measuring together up to 1.5 cm. in width. The kidney pelvis was dilated and lined with a moderately congested mucosa. The renal artery showed no abnormality. The right kidney was extremely reduced in size, weighing only 10 grams.

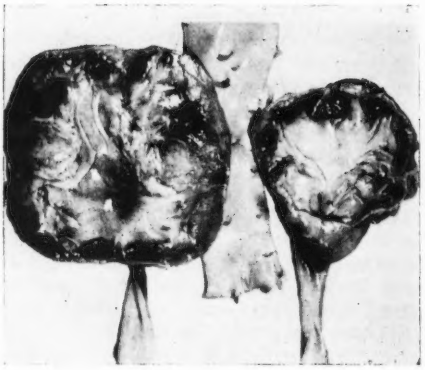

FIGURE 2

Bilateral hydronephrosis and pyelonephritis. The parenchyma of the small right kidney is reduced to a thin shell.

On section the kidney parenchyma was reduced to a thin shell of tissue in which a differentiation between cortex and medulla could not be made. This tissue measured in its thickest area only 0.5

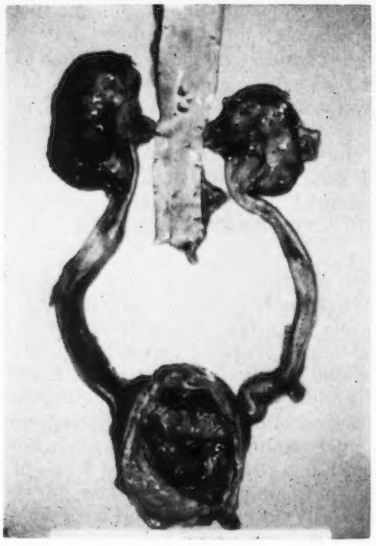

FIGURE 3

Bilateral pyelonephritis and hydroureter. Cystitis. Note the thickening of the walls of the dilated ureters.

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cm. in thickness (Fig. 2). The kidney pelvis was dilated and lined with congested mucosa. The right renal artery was much smaller than normal, measuring only 0.2 cm. in its outside diameter (Fig. 1). This would indicate that in addition to bilateral destructive kidney disease we have a congenital hypoplasia of the right kidney as indicated by its smallness and the associated smallness of the right renal artery.

It is conceivable that the abnormal x-ray findings of the right kidney pelvis on retrograde pyelography were due to this congenital abnormality. In addition to bilateral dilatation of the kidney pelvis there was bilateral hydroureter (Fig. 3). Microscopically we had no difficulty in identifying the pathologic features of the

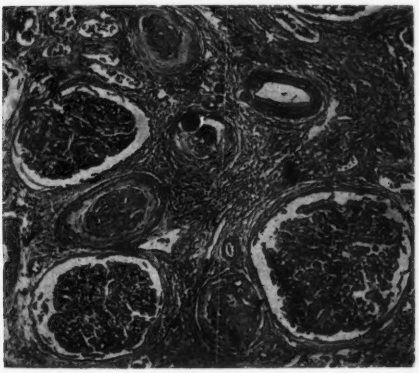

FIGURE 4

Pyelonephritis. There are hyalinized glomeruli as well as others with intact glomerular tufts and marked pericapsular fibrosis. H&E x80.

acquired disease as pyelonephritis. The histologic diagnosis of pyelonephritis was made because in addition to hyalinized glomeruli we found relatively intact glomerular tufts with varying degrees of pericapsular fibrosis. (Fig. 4.) This disparity between the preservation of the glomerular tufts and the pericapsular fibrosis is charactertstic of pyelonephritis in contrast to chronic glomerulonephritis in which the glomerular tuft is primarily affected.

In addition there was marked infiltration of the interstitial tissue of the kidney

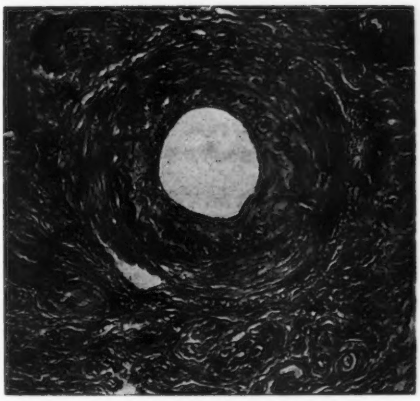

FIGURE 5

Marked arteriosclerosis associated with pyelonephritis. H&E x115.

with chronic inflammatory cells. Both arteriolo- and arteriosclerosis (Fig. 5) were present; these vascular changes were interpreted as being secondary to the pyelonephritis or the associated hypertension or both. In several areas there was indication of a persistent suppurative process with the tubules containing casts composed of polymorphonuclear leukocytes and necrotic debris. (Fig. 6.)

On the basis of these findings we made a diagnosis of chronic suppurative pyelonephritis indicating both the long stand-

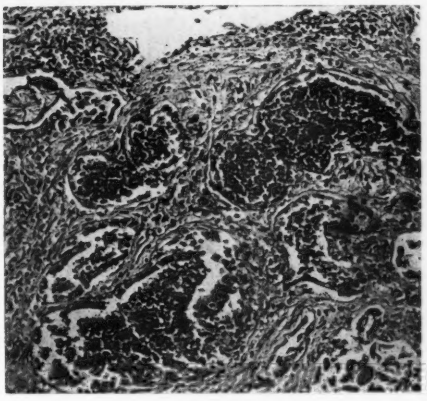

FIGURE 6

Suppurative pyelonephritis. Casts composed of polymorphonuclear leukocytes and necrotic debris within distended tubules. H&E x140.

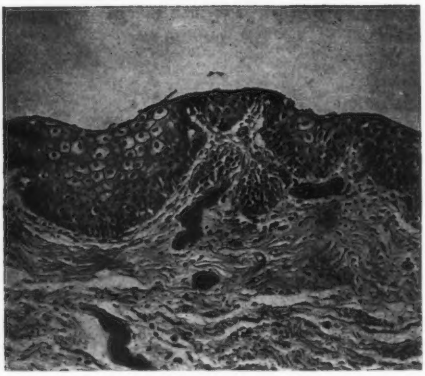

FIGURE 7

Urinary bladder. Chronic cystitis with squamous metaplasia of the epithelial lining. H&E x125.

ing nature of the disease as well as the fact that we had actively progressive disease until the time of death. Sections from the kidney pelves, the ureters, and the urinary bladder showed chronic inflammation and fibrosis involving the walls of these organs. The transitional epithelial lining of the urinary bladder showed in some areas squamous metaplasia (Fig. 7) as we find in chronic inflammatory and irritative processes. Outside the genitourinary system we found a slight but definite hypertrophy of the heart, which weighed 350 grams; the hypertrophy was primarily due to thickening of the left ventricular wall which measured up to 1.7 cm. This is entirely consistent with the mild hypertension which this patient had in the course of her illness.

There was also a secondary hyperplasia of the parathyroids, three of which were identified and had a combined weight of 0.3 grams. Microscopically they showed a diffuse cellularity with almost complete absence of interstitial fat. This finding is an indication of renal insufficiency of long standing. Finally, we found a terminal bronchopneumonia of both lower lobes.

In summary, the final anatomical diagnoses were as follows: 1) Chronic pyelonephritis (bilateral); 2) hydronephrosis (bilateral); 3) hypoplasia of the right kidney and right renal artery; 4) arteriosclerosis and arteriolosclerosis of kidneys; 5) hydroureter, bilateral; 6) chronic cystitis and ureteritis; 7) hypertrophy of heart (slight); 8) bronchopneumonia of both lower lobes; 9) hyperplasia, secondary, of parathyroids.

The clinical course of this patient's illness is entirely consistent with the post mortem findings of chronic cystopyelonephritis with severe destructiotn of kidney parenchyma in a patient who had congenital hypoplasia of one kidney to begin with. However, some of you might find it hard to explain why a patient without anatomical evidence of obstruction of the urinary passages should develop hydronephrosis and pyelonephritis of such severity.

The generally accepted explanation of this phenomenon is that there is, in pregnancy, an almost physiological hydonephrosis beginning with the fifth month and lasting two or three weeks after delivery. As a rule hydronephrosis does not persist unless there are complications. However, when there is an association with repeated infection of the walls of the urinary passages, particularly of the ureters, the ureteral walls become infiltrated with inflammatory cells, and although there is no anatomical obstruction to the urinary flow, a physiological obstruction develops due to loss of peristalsis by the damaged ureters which no longer propel the urine as they normally would.

This interference of the chronic inflammation with the normal function of the urinary passages may lead to a persistent hydronephrosis rather than a temporary one as it occurs in normal pregnancy, and increasingly severe infection and inflammation may lead to irreversible changes in the walls of the ureters, the kidney pelves, and in the kidney itself. In addition, neuromuscular abnormalities of a functional type which cannot be demonstrated microscopically have also been postulated to explain the presence of hydroureter and hydronephrosis in the absence of anatomical obstruction of the urinary passages.

Dr. Isaacs: I must compliment Dr. Kadin on his work and his diagnosis, but I am somewhat disappointed that he did not mention the hyperplasia of the parathyroids.

Dr. Kadin: Perhaps I did not miss the hyperplasia of the parathyroids on this basis; the attempt on my part was to make a diagnosis of the basic disease and not the other phenomena which occur with the disease. Had I wanted to go into a discussion of the pathology that is found in chronic renal disease, it would have been necessary to discuss hyperplasia of the parathyroids. In fact, many other phenomena may be discussed that occur in the pathophysiology of uremia.

This case does bring up one thing that many of us do not realize and that is the effect of pregnancy on renal disease. One has to be very wary in any patient with any mild degree of renal disease, to watch them very closely with pregnancy, for if there are signs of progression of renal impairment, to advise no further pregnancies. I think that this was the big mistake in this case. Had this woman had but two pregnancies I think that she would have had a relatively normal life span, but the insults attended upon by two more pregnancies were sufficient to cause further renal disease and her death.

BOOK REVIEWS

PEDIATRIC CARDIOLOGY; Alexander S. Nadas, M.D., F.A.A.P.; W. B. Saunders Co., Philadelpia and London, 1957.

The author is Assistant Clinical Professor of Pediatrics, Harvard Medical School; Cardiologist, The Children's Hospital; Physician, Sharon Cardivascular Unit, Children's Medical Center, Boston.

The volume does not pose as a reference text on the material but rather intends itself as a handbook for the pediatrician, general physician, and medical student. Emphasis is placed on clinical recognition and management of heart disease in children. Technique of catheterization and angiocardiography, for example, have been described, but primary emphasis has been kept on the principle and procedure of clinical management. There is an extensive list of references and judicious use of clean, well reproduced illustrations. The author has called upon his ten years of experience at the Children's Medical Center. Diseases and even important therapeutic measures such as open heart surgery and hypothermia, with which he had had little personal contact, are discussed only cursorily.

The book is warmly recommended to all who wish to read an up-to-date, well-written, easily-read discussion on this topic which is growing daily in significance.

B. W. N.

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CLINICAL USE OF RADIOISOTOPES by W. H. Beierwaltes, M.D.; P. C. Johnson, M.D.; and A. J. Solari, M.D. Cloth, 1st Edition, 584 pages with 204 illustrations. Philadelphia: W. B. Saunders Co., 1956. $11.50.

This is a new book, dealing with the use of currently available isotopes in the daily practice of medicine. The authors give all the necessary fundamentals and background material such as what one should know about radioactivity; the requirements for a physician's use of radioisotopes; detection of radiation, scintillation counters, etc. Detailed and comprehensive sections describe the instrumentation and equipment needed in the radioisotope unit, plus an outline of how to set up and manage a radioisotope unit.

There is the latest information on: Cardiac output determinations using radioisotopes; methods for diagnosis of gastrointestinal malabsorption syndromes; scintillation localization of brain tumors; latest techniques for blood volume determinations; use of radioisotopes in differential diagnosis of anemias. There are two additional chapters devoted to Biological Effects of Radiation and Health Precaution in Use of Radioisotopes.

L. W. D.

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CLINICAL EXAMINATIONS IN NEUROLOGY by members of the sections of Neurology and Physilogy, Mayo Clinic and Mayo Foundation. 1st Edition. 370 pages. Philadelphia: W. B. Saunders Co., 1956.

This is a book written by sixteen Neurologists of the Mayo Clinic. It is a compact and practical book. The men discuss the various phases of Neurology and combine with this the anatomy and physiology required to understand the tests. There are many helpful hints included to help the medical student and the general practitioner. In addition, sketches are used to aid in understanding.

An excellent chapter is on the differential diagnosis of coma, including: Acute Alcoholic Intoxication, Acute Barbiturate Intoxication, Bromide Intoxication, Carbon Monoxide Poisoning, Acute Salicylate Intoxication, Acute Arsenic and Lead Intoxication and Acute Morphine Intoxication.

S. H. C.

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THE PHYSICIAN-WRITER'S BOOK—TRICKS OF THE TRADE OF MEDICAL WRITING by Richard M. Hewitt, A.M., M.D.; W. B. Saunders Co., Philadelphia and London, 1957.

The author is Senior Consultant, Section of Publications, The Mayo Clinic; Associate Professor of Medical Literature, The Mayo Foundation, Graduate School, University of Minnesota.

As witnessed by the title, the book is written in a light, pleasant manner — making palatable a dissertation on syntax. He considers unity, coherence, and emphasizes the characteristics of good writing, whether the structural unit under consideration is the sentence, the paragraph or the entire composition. He presumes that the book will be used as a manual rather than be read from beginning to end. He uses many examples of good writing and examples of undesirable or erroneous material that he had found in medical and general literature.

It seems hardly necessary to remark that this book would be attractive only to those who plan to add their names to the roles of medical research and to be contributors to medical literature. To those, it is highly recommended as an interesting and lucrative experience.

B. W. N.

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TREATMENT OF HEART DISEASE by Harry Gross, M.D., F.A.C.P.; and Abraham Jezer, M.D. Cloth. First Edition. 549 pages. Philadelphia: W. B. Saunders Co., 1956. $15.00.

This text is a presentation of therapy in heart disease based upon sound physiological principles. It is the authors' intention to reach the general physician who, with understanding of the physiologic mechanisms involved, may more readily understand the symptoms and clinical course of his patients.

This volume is divided into seven parts and an appendix. The first part consists of cardiac physiology; remaining sections deal with degenerative diseases, inflammation of the heart or the results of infection, congenital anomalies, effects of pregnancy and surgery on the heart, effects of metabolic disturbance and vitamin deficiencies on heart disease, and finally, the adjustment to emotional disturbances and the problem of rehabilitation.

Present-day management of congestive failure, both in its acute and in its chronic form, and the complicating electrolyte imbalance are explored. Management of the arrythmias, uses of digitalis; quinidine, mercurials, and diets are also fully discussed. In the section on degenerative diseases is included an excellent chapter on hypertension including the recently developed antihypertensive blocking agents. Other noteworthy chapters are: arteriosclerotic heart disease (medical and surgical management); prophylactic and active treatment of rheumatic heart disease, including a detailed discussion of criteria for surgical intervention; latest treatment and diagnostic technique of congenital heart disease; effects of anesthetic agents on cardiac function; and psychosomatic aspects of heart disease.

Numerable illustrations graphically present many of the points which are stressed in the text. This book should prove a valuable adjunct both to the medical student and physician for a comprehensive understanding of all the major aspects of heart disease.

L. W. D.

THE RECOVERY ROOM by Max S. Sadove, M.D., and James H. Cross, M.D. 1st Edition. 597 pages. Philadelphia: W. B. Saunders Co., 1956.

This book concerns a subject which has become increasingly more important to the medical field in recent years. It is the first of its type to be published. The book is written by an impressive array of Chicago physicians.

The book concerns itself with pre-operative management (since post-operative care is related to pre-operative care), post-operative management of the routine and complicated cases, and principles of recovery room management. There are excellent discussions of the use and methods of giving various anethesias, diagnosis and treatment of surgical shock as well as post-operative orders and their rationale. Each chapter is written by an authority in his field.

The book is small but very complete. Drawings and charts are used freely to aid in understanding. The book is well worth reading, whether the reader be a medical clerk, general practitioner, internist or physician.

S. H. C.

ABSTRACTS SECTION

SAIDEL, LEO J. *(Asst. Prof. Biochemistry):* Ultraviolet Absorption Spectra of Peptides. II. Glycine Peptides Containing More Than One Substituted Amide Link. Arch. of Biochem. and Biophys., *56:* 45-58, 1955.

Spectra in the ultraviolet region from about 200 to 240 mμ are presented for aqueous solutions at various pH values for H. Glym. OH, where n = 2, 3, 4, 5, and 6, for glycine anhydride, H. Ala-Gly_2. OH, H. Leu-Gly_2. OH, Av-Phe-Gly. OH, and Ac-Phe-Gly_2. OH. Also, the spectrum of a preparation of polyglycine methyl ester in 43% H_3PO_4 is presented.

Evidence is presented to show that the spectrum of an individual peptide link within a peptide containing more than one peptide link is influenced by the presence of adjacent peptide links.

The attempt is made to show that it may be possible to assign to each of the endoresidues in a short peptide chain individual residue spectra which are additive no matter what the sequence of residues is in the chain. The rational basis for this hypothesis is discussed. The main evidence for it is based upon the observation that at pH's 1 and 6 there appears to be an approximately constant difference between the spectra of a number of pairs of peptides which differ from each other only by a single endoglycine residue. Extension of the above principle to macromolecules is briefly discussed.

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SEIDEL, LEO J. *(Asst. Prof. Biochemistry):* Ultraviolet Absorption Spectra of Peptides. III. N, N-Dialkylamides Including Polyvinylpyrrolidone. J. of Amer. Chem. Soc., *77:* 3892-3895, 1955.

The ultraviolet absorption spectra of aqueous solutions of the N, N-dialkylamides: N, N-dimethylacetamide, N, N-diethylacetamide, acetyl-L-proline and glycyl-L-proline in the various ionic forms, 1-ethyl-2-pyrrolidone, and polyvinylpyrrolidone (PVP) in the region from 200 to 240 mu, all suggest an absorption maximum slightly below 200 mμ. The exact location and intensity of this band depends to some extent upon the nature of the substitute on the amide group.

Contrary to the findings of others, the spectrum of PVP does not exhibit maxima above 200 mμ and does not vary appreciably with concentration or pH (6.2-12.2). Below 224 mμ, three preparations of PVP of markedly different molecular weight (25,000-251,000) exhibited practically identical spectre, which were lower than that of 1-ethyl-2-pyrrolidone.

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SAIDEL, LEO J. *(Asst. Prof. Biochemistry):* Ultraviolet Absorption Spectra of Peptides. I. Compounds Containing a Single Monoalhyl-Substituted Amide Link. Arch. of Biochem. and Biophys., *13:* 184-200, 1955.

Spectra in the ultraviolet region from about 200 to 240 mμ are represented for aqueous solutions of the collowing compounds at various pH values: (a) acetyl and/or glycyl derivatives of nearly all of the amino acids which commonly occur in proteins; (b) a number of isomeric dipeptides with the sequence of the residues reversed; and (c) glycine amide, pyrrolidone carboxylic acid, and N-monomethylacetamide.

The attempt is made to consider the spectrum of a dipeptide as the resultant of interactions between the peptide link and other groups which may occur on the molecule, *viz.,* a terminal carboxyl group, a terminal amino group, and various specific amino acid side chains.

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POZZA, GUIDO; GALANSINO, GIORGIO; and FOA, PIERO P.: Insulin Secretion Following Carbutamide Injections in Normal Dogs. Proc. Soc. Exp. Biol. Med., *93:* 539, 1956.

The mechanism of the hypoglycemic action of carbutamide in normal dogs was studied by means of pancreatic-femoral and mesentric-femoral cross-circulation experiments. The results of these acute experiments suggest that: 1.) carbutamide hypoglycemia is accompanied by the secretion of insulin; 2.) carbutamide does not cause degranulation or other significant changes in the appearance of pancreatic A and B cells; 3.) the clearance of carbutamide from the blood is relatively slow; and 4.) some dogs are refractory to the hypoglycemic action of carbutamide in doses of 50 mg/kg I.V.

The results are consistent with the hypothesis that insulin secretion is one of the results of carbutamide action. The continued administration of carbutamide in animals and men with reduced pancreatic reserve may stimulate the islets of Langerhans to regeneration or to exhaustion. Although some evidence suggests that islet growth does occur, the second alternative has not been ruled out and should be investigated before the sulfonylureas can be recommended for the prolonged treatment of diabetes mellitus.

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DASLER, WALDEMAR: Protective Action of Glutamine Cysteine and Other Substances Against Experimental Lathyrism in the Rat. Proc. Soc. Exper. Biol. and Med., 1956, v. 91, pp. 554-557.

Through the toxication of sweet peas, Lathyrus odoratus is known to be due to B-aminopropionitrile, the specific metabolic disturbance in the rat is still undetermined. The finding of a protective agent may also be of considerable medical importance because of the possible relationship between odoratism (sweet pea lathyrism) and a number of human diseases of unknown etiology.

None of the supplements tested for their protective action gave complete protection. Only L-cysteine-HCL, L-glutamine, and proteins (casein, gelatin, lactalbumin, and casein hydrolysate)

were partially protective. It was also noted that the protection afforded by L-cysteine-HCL, is prolonged if the diet is made up fresh weekly. Also, when the diets are mixed fresh weekly, cysteine plus glutamine gave greater protection than does either supplement alone.

B. W. N.

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DASLER, WALDEMAR: Production of Experimental Lathyrism in the Rat by Two Different Beta-substituted Ethylamines. Proc. Soc. Exper. Bio. and Med., 1955, v. 88, pp. 196-199.

Crystalline glutamyl aminopropionitrile has been isolated from the seed of the flowering sweet pea (Lathyrus adoratus). When it is fed to rats it produces skeletal changes typical of odoratism, the experimental lathyrism produced in rats by the feeding of sweet peas. Crystals isolated from the sweet pea were found to have the same infra-red spectrum as synthetic B-aminopropionitrile sulphate. Both Baminopropionitrile and B-mercaptoethylamine produced skeletal changes similar to those caused by sweet peas. It appears that neither the B-aminoethyl group nor the cyano group are essential for the production of this type of lesion.

B. W. N.

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GOLDFARB, A. R.; MELE, A.; and GUTSTEIN, N. *(Dept. of Biochemistry):* Basicity of the Amide Bond. J. Am. Chem. Soc., *77:* 6194, 1955.

During the course of an investigation of the ultraviolet absorption spectra of amides and repitides, it was observed that these compounds showed a marked lowering of the molar extinction coefficient in concentrated sulfuric acid solutions. The authors have made a study of a series of acetamide derivatives, using a spectrophotometric method, to determine the effect of substituents on the nitrogen and of adjacent charge on the basicity of the amide bond.

The value of P.K., which was found for acetamide, is in agreement with previously reported values. The basicity of the amide bond may be considered to be associated with the resonating forms.

The authors are continuing the study for simple and complex peptides and proteins and will report further studies in the near future.

This work was supported in part by Grant #NSF-G617 from the National Science Foundation.

S. H. C.

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GOLDFARB, A. R.; LIBRETTI, A.; KAPLAN, M.; ABRAMSON, H. A.; and AARONSON, A. *(Dept. of Biochemistry and The Allergy Clinic of Dept. of Medicine):* Preparation and Properties of a Single Antigen from Giant Ragweed Pollen. Int. Arch. of Allergy and Applied Immunology, *8:* 243, 1956.

The authors hypothesized that there is one antigen responsible for the allergy caused by giant ragweed pollen and that other antigens were chemically associated to the "master antigen." Giant ragweed pollen was fractionized into five parts. One fraction (J) is thought to be the active antigen, while another fraction (K) is a mixture of the other antigens.

This work was supported in part by the R. B. Smith Fund, the Asthmatic Children's Aid of Chicago and the Josiah Macy, Jr., Foundation.

S. H. C.